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(54) Title: DNA MOLECULES ENCODING HUMAN NHL, A DNA HELICASE

(57) Abstract: The present invention disclosed isolated nucleic acid molecules (polynucleotides) which encode NHL, a putative DNA helicase. The present invention in turn relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding NHL, substantially purified forms of associated NHL, associated mutant proteins, and methods associated with identifying compounds which modulate NHL, which will be useful in the treatment of various neoplastic disorders. Both a genomic clone containing regulatory and intron sequences, as well as the exon structure and open reading frame of human NHL are disclosed.

5

TITLE OF THE INVENTION

DNA MOLECULES ENCODING HUMAN NHL, A DNA HELICASE

10 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional application 60/169,970 filed December 9, 1999.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

15 Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

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FIELD OF THE INVENTION

The present invention relates in part to isolated nucleic acid molecules (polynucleotides) which encode NHL, a putative DNA helicase. The present invention also relates to recombinant vectors and recombinant hosts which contain a

25 DNA fragment encoding NHL, substantially purified forms of associated NHL, associated mutant proteins, and methods associated with identifying compounds which modulate NHL, which will be useful in the treatment of various neoplastic disorders, given that this gene is located at 20q13.3 and immediately adjacent to M68/DcR3, which is involved in tumor growth. Also included within the present

30 invention is a human genomic fragment representing this portion of the human genome, along with three additional genes (M68/DcR3, SCLIP, and ARP).

BACKGROUND OF THE INVENTION

Naumovski et al. (1985, *Mol. Cell Biol.* 5:17-26; Reynolds et al. (1985 *Nucleic*
5 *Acid Res* 13:2357-2372) and Weber et al. (1990 *EMBO J.* 9:1437-1447) disclose
members of the RAD3/ERCC2 gene family of DNA helicases.

It is known that several chemotherapeutic agents inhibit helicases, including
actinomycin C1, daunorubicin and nogalamycin (Tuteja, et al., 1997, *Biochem.*
Biophys. Res. Comm. 236(3):636-640), and a prostate cancer drug, CI-958 (Lun, et
10 al., 1998, *Cancer Chemother. Pharmacol.* 42(6):447-453). In addition, some
topoisomerases have been shown to have anti-cancer activity.

Despite the identification of the aforementioned helicase-encoding genes and
chemotherapeutic agents, it would be advantageous to identify additional genes which
reside within chromosomal regions associated with a disease state such as cancer as
15 well as a gene which encodes a type of protein which may be associated with that
disease. The present invention addresses and meets this need by disclosing a DNA
molecule encoding a DNA helicase with a chromosomal location suggestive of
association with cancer.

20 SUMMARY OF THE INVENTION

The present invention relates to an isolated or purified nucleic acid molecule
(polynucleotide) which encodes a novel mammalian DNA helicase.

The present invention also relates to an isolated nucleic acid molecule
(polynucleotide) which encodes mRNA which expresses a novel human DNA
25 helicase, NHL.

A preferred aspect of the present invention relates to an isolated or purified
DNA molecule which encodes human NHL, the nucleotide sequence as set forth in
Figure 1A-B and SEQ ID NO:1.

The present invention also relates to biologically active fragments or mutants
30 of SEQ ID NO:1 which encode a mRNA molecule expressing a novel DNA helicase,
NHL. Any such biologically active fragment and/or mutant will encode either a
protein or protein fragment which at least substantially mimics the biological
properties of the human NHL protein disclosed herein in Figure 2 and as set forth as
SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to

nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a functional NHL protein in a host cell, so as to be useful for screening for agonists and/or antagonists of NHL activity.

5 The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification.

 The present invention also relates to a substantially purified form of a human NHL protein which comprises the amino acid sequence disclosed in Figure 2 and set
10 forth as SEQ ID NO:2.

 A preferred aspect of this portion of the present invention is a NHL protein which consists of the amino acid sequence disclosed in Figure 2 and set forth as SEQ ID NO:2.

 Another preferred aspect of the present invention relates to a substantially
15 purified NHL protein, preferably a human NHL protein, obtained from a recombinant host cell containing a DNA expression vector comprises a nucleotide sequence as set forth in SEQ ID NO:1 and expresses the respective NHL protein. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line.

20 The present invention also relates to biologically active fragments and/or mutants of a NHL protein comprising the amino acid sequence as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or
25 prophylactic use and would be useful for screening for selective modulators, including but not limited to agonists and/or antagonists for human NHL pharmacology.

 A preferred aspect of the present invention is disclosed in Figure 2 and is set forth as SEQ ID NO:2, a respective amino acid sequence which encodes human NHL. Characterization of one or more of these DNA helicase-like proteins allows for
30 screening methods to identify novel NHL modulators that may be useful in the treatment of human neoplastic disorders. The modulators selected through such screening and selection protocols may be used alone or in conjunction with other cancer therapies. As noted above, heterologous expression of a NHL protein will allow the pharmacological analysis of compounds which modulate NHL activity and

hence may be useful in various cancer therapies. To this end, heterologous cell lines expressing a NHL protein can be used to establish functional or binding assays to identify novel NHL modulators.

The present invention also relates to polyclonal and monoclonal antibodies
5 raised in response to either the NHL or a biologically active fragment of NHL.

The present invention relates to transgenic mice comprising altered genotypes and phenotypes in relation to NHL and its *in vivo* activity.

The present invention also relates to NHL fusion constructs, including but not limited to fusion constructs which express a portion of the NHL protein linked to
10 various markers, including but in no way limited to GFP (Green fluorescent protein), the MYC epitope, and GST. Any such fusion constructs may be expressed in the cell line of interest and used to screen for NHL modulators.

Therefore, the present invention relates to methods of expressing mammalian NHL, and preferably human NHL, biological equivalents disclosed herein, assays
15 employing these gene products, recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these assays which act as agonists or antagonists of NHL activity.

The present invention also relates to the isolated genomic sequence which comprises SEQ ID NO:1, a 115 kb genomic fragment set forth herein as SEQ ID
20 NO:3. As especially preferred aspect of this portion of the invention is the region of the genomic fragment of SEQ ID NO:3 which comprises the regulatory and coding regions of human NHL, as well as intervening sequences (introns). This 115 kb fragment contains at least the coding region of four genes, NHL, M68/DcR3, SCLIP and ARP. As discussed herein, it has been shown that this region of chromosome 20
25 is associated with tumor growth. Therefore, an aspect of this invention also comprises the use of one or more regions of this 115 kb genomic sequence to identify compounds which up or downregulate expression of one or more of the genes localized within this 115 kb region, wherein this up or down regulation results in an interference of tumor growth. For example, a transcription element of one of these
30 four genes may be responsible for M68/DcR3 (and/or NHL) overexpression in tumors, and if M68 or NHL overexpression in tumors has a caustic role, blockage of M68/DcR3 or NHL overexpression in tumors by interfering with this transcription site will be useful.

It is an object of the present invention to provide an isolated nucleic acid molecule (e.g., SEQ ID NO:1) which encodes novel form of human NHL, or fragments, mutants or derivatives of human NHL as set forth in Figure 2 and SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to nucleotide
5 substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators of human NHL activity.

It is a further object of the present invention to provide the mammalian, and
10 especially human, NHL proteins or protein fragments encoded by the nucleic acid molecules referred to in the preceding paragraph.

It is a further object of the present invention to provide recombinant vectors and recombinant host cells which comprise a nucleic acid sequence encoding mammalian, and especially human, NHL protein and biological equivalent thereof.

15 It is an object of the present invention to provide a substantially purified form of human NHL, as set forth in Figure 2 and SEQ ID NO:2.

Is another object of the present invention to provide a substantially purified recombinant form of a NHL protein which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and
20 appropriately expresses a complete open reading frame as set forth in SEQ ID NO:1, resulting in a functional, processed form of NHL. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line.

It is an object of the present invention to provide for biologically active fragments and/or mutants of mammalian, and especially human, NHL, such as set
25 forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic and/or prophylactic use.

It is also an object of the present invention to use NHL proteins or biological
30 equivalent to screen for modulators, preferably selective modulators, of human NHL activity. Any such compound may be useful in screening for and selecting compounds active against human neoplastic disorders.

As used herein, "substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of

other nucleic acids. Thus, a human NHL DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-NHL nucleic acids. Whether a given
5 NHL DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

As used herein, "substantially free from other proteins" or "substantially
10 purified" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a NHL protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-NHL proteins. Whether a given
15 NHL protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting. As used interchangeably with the terms "substantially free from other proteins" or "substantially purified", the
20 terms "isolated NHL protein" or "purified NHL protein" also refer to NHL protein that has been isolated from a natural source. Use of the term "isolated" or "purified" indicates that NHL protein has been removed from its normal cellular environment. Thus, an isolated NHL protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does
25 not imply that an isolated NHL protein is the only protein present, but instead means that an isolated NHL protein is substantially free of other proteins and non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the NHL protein *in vivo*. Thus, a NHL protein that is recombinantly expressed in a prokaryotic or eukaryotic cell and substantially purified from this host cell which does not
30 naturally (*i.e.*, without intervention) express this protein is of course "isolated NHL protein" under any circumstances referred to herein. As noted above, a NHL protein preparation that is an isolated or purified NHL protein will be substantially free from other proteins will contain, as a percent of its total protein, no more than 10%,

preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-NHL proteins.

As used interchangeably herein, "functional equivalent" or "biologically active equivalent" means a protein which does not have exactly the same amino acid sequence as naturally occurring NHL, due to alternative splicing, deletions, mutations, substitutions, or additions, but retains substantially the same biological activity as NHL. Such functional equivalents will have significant amino acid sequence identity with naturally occurring NHL and genes and cDNA encoding such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring NHL. For example, a naturally occurring NHL disclosed herein comprises the amino acid sequence shown as SEQ ID NO:2 and is encoded by SEQ ID NO:1. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide level to SEQ ID NO:1.

As used herein, "a conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid).

As used herein, the term "mammalian" will refer to any mammal, including a human being.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-B shows the nucleotide sequence which comprises the open reading frame which encodes human NHL, the nucleotide sequence set forth as SEQ ID NO:1. The initiating Met residue (ATG) and the stop codon (TAG) are underlined.

Figure 2 shows the amino acid sequence of human NHL as set forth in SEQ ID NO:2.

Figure 3 shows the alignment of amino acid sequences of human NHL to ERCC2/RAD3 gene family members. Rep D (*Dictyostelium discoideum*); RAD 3 (*S. cerevisiae*); RAD15 (*S. pombe*) and XP_GroupD (*Homo sapien*).

Figure 4 shows Northern analysis of NHL expression in multi-human tissues.

Figure 5A-B show the genomic structure of the NHL gene (Figure 5A) and the entire 115 kb genomic region (Figure 5B) containing the NHL, M68/DcR3, SCLIP

and ARP genes.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated or purified nucleic acid molecule
5 (polynucleotide) which encodes a novel mammalian DNA helicase. An especially
preferred aspect of this invention relates to an isolated nucleic acid molecule
(polynucleotide) which encodes mRNA which expresses a novel human DNA
helicase, NHL.

The gene M68/DcR3 is a secreted TNFR member that is overexpressed in a
10 number of human tumors. M68/DcR3 is located at 20q13.3, a known site that is
associated with frequent gene amplification in cancer. M68/DcR3 protein binds to
FASL and inhibit FAS mediated apoptosis. Thus, genes tightly linked to M68/DcR3
may be coregulated (e.g. co overexpressed and/or amplified in tumors). During the
course of cloning the genomic M68/DcR3 fragment and identifying genes that are
15 linked to M68/DcR3 at 20q13.3, three genes, including a novel gene that is similar to
the Rad3/ERCC2 helicase family, were identified (termed NHL) in the immediately
adjacent (overlapping) region. Given NHL's chromosomal location and the frequent
association of DNA helicases with human genetic disorders (mutations in DNA
helicases have been found associated with multiple diseases, including xeroderma
20 pigmentosum, Cockayne's syndrome, Bloom's syndrome, and Werner's syndrome),
NHL is a candidate for contribution to certain human neoplastic disorders. To this
end, the genomic clone for this gene is disclosed and the complete sequence is
determined. The transcript was identified through exon prediction using GRAIL2 and
sequence alignment to a contiguous 4.5 kilobase region of chromosome 4 (88%
25 sequence identity). The complete exon structure of NHL was subsequently confirmed
by RT-PCR analysis. Multiple sequence alignment of NHL to known helicases
showed that NHL contains all the seven critical helicase domains. BLAST analysis of
the predicted 1,219 amino acid sequence revealed an approximately 26% sequence
identity and 48% sequence similarity to the RAD3/ERCC2 gene family of DNA
30 helicases (Naumovski et al., 1985 *Mol. Cell Biol.* 5:17-26; Reynolds et al., 1985
Nucleic Acid Res 13:2357-72; Weber et al., 1990 *EMBO J.* 9:1437-1447). The
mRNA expression pattern of NHL was also examined in multiple human tissues.
Radiation hybrid chromosomal mapping reconfirms that it is linked to M68/DcR3
locus.

A preferred aspect of the present invention relates to an isolated or purified DNA molecule which encodes human NHL, the nucleotide sequence as set forth in Figure 1A-B and SEQ ID NO:1, which is as follows:

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AGTCAGCCCT GCTGCCAGCC AGTGCCGGGT GCTGGGGACT CAGGGAGGCC CGCCGGGACC
5  ACTGCGGGAC AGTGAGCCGA GCAGAAGCTG GAACGCAGGA GAGGAAGGAG AGGGGGCGGT
CAGGGCTCTC AGGAGCCGGG TCCTGGGCAA GGCGCAGCCG TTTTCAAATT TTCAGGAAAG
CGGTCTGGCTC AACTCTGAGC AGTAAAAAGA TGCCTCTGGG GAGGAGGCCG GTGCAGCTCT
CCGGGCAATG GTGGTGGCTC GGCTTAGAGA GGCGGTAGTG GAACGCAGAC CCTGGTGGGG
GAATGACATC AAGGGAGGAG ACGGGCGGGA CCCCAGATTT CTGCCTGTGG GCGATGGAAG
10 TGAGGTTCAC TGGCCAGCGG AGCCGGACAC AGAACGCGCA AAACGCCGTG TAGGCCCTGGA
GGAGCCGAAG AGCAGGCGGA CCCCCTCCGC GGGGGAACAG TTTCCGCCGG GAGCACAAAG
CAACGGACCG GAAGTGGGGG GCGGAAGTGC AGTGGGCTCA GCGCCGACTG CGCGCTCTG
CCCCGAAAA CTCTGAGCTG GCTGACAGCT GGGGACGGGT GGCGGCCCTC GACTGGAGTC
GGTTGAGTTC CTGAGGGACC CCGTTCTTGG AAGGTCGCC GCGGAGACAA GTGAGCAGTC
15 TGTGCCATAG GGATTCTCGA AGAGAACAGC GTTGTGTCCC AGTGCACATG CTCGCATCGC
TTACCAGGAG TGCCCCGAGC CCTAAGATGT TCGGAGTGGT TTTTTCGCAC AGACCCGAAT
AGCCTGCCCC TCAGCCACGC TCTGTGCCCT TCTGAGAACA GGCTGATATG CCAAGATAG
TCCTGAATGG TGTGACCGTA GACTTCCCTT TCCAGCCCTA CAAATGCCAA CAGGAGTACA
TGACCAAGGT CCTGGAATGT CTGCAGCAGA AGGTGAATGG CATCCTGGAG AGCCCTACGG
20 GTACAGGGAA GACGCTGTGC CTGCTGTGCA CCACGCTGGC CTGGCGAGAA CACCTCCGAG
ACGGCATCTC TGCCCGCAAG ATTGCCGAGA GGGCGCAAGG AGAGCTTTTC CCGGATCGGG
CCTTGTCATC CTGGGGCAAC GCTGCTGCTG CTGCTGGAGA CCCCATAGCT TGCTACACGG
ACATCCCAAA GATTATTTAC GCCTCCAGGA CCCACTCGCA ACTCACACAG GTCATCAACG
AGCTTCGGAA CACCTCCTAC CGGCCTAAGG TGTGTGTGCT GGGCTCCCGG GAGCAGCTGT
25 GCATCCATCC TGAGGTGAAG AAACAAGAGA GTAACCATCT ACAGATCCAC TTGTGCCGTA
AGAAGGTGGC AAGTCGCTCC TGTCATTTCT ACAACAACGT AGAAGAAAA AGCCTGGAGC
AGGAGCTGGC CAGCCCCATC CTGGACATTG AGGACTTGGT CAAGAGCGGA AGCAAGCACA
GGGTGTGCCC TTACTACCTG TCCCGGAACC TGAAGCAGCA AGCCGACATC ATATTATGC
CGTACAATTA CTTGTTGGAT GCCAAGAGCC GCAGAGCACA CAACATTGAC CTGAAGGGGA
30 CAGTCGTGAT CTTTGACGAA GCTCACAACG TGGAGAAGAT GTGTGAAGAA TCGGCATCCT
TTGACCTGAC TCCCCATGAC CTGGCTTCAG GACTGGACGT CATAGACCAG GTGCTGGAGG
AGCAGACCAA GGCAGCGCAG CAGGGTGAGC CCCACCGGA GTTCAGCGCG GACTCCCCCA
GCCCAGGGCT GAACATGGAG CTGGAAGACA TTGCAAAGCT GAAGATGATC CTGCTGCGCC
TGGAGGGGGC CATCGATGCT GTTGAGCTGC CTGGAGACGA CAGCGGTGTC ACCAAGCCAG

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GGAGCTACAT CTTTGAGCTG TTTGCTGAAG CCCAGATCAC GTTTCAGACC AAGGGCTGCA
 TCCTGGACTC GCTGGACCAG ATCATCCAGC ACCTGGCAGG ACGTGCTGGA GTGTTACCA
 ACACGGCCGG ACTGCAGAAG CTGGCGGACA TTATCCAGAT TGTGTTCACT GTGGACCCCT
 CCGAGGGCAG CCCTGGTTCC CCAGCAGGGC TGGGGGCCCT ACAGTCCTAT AAGGTGCACA
 5 TCCATCCTGA TGCTGGTCAC CGGAGGACGG CTCAGCGGTC TGATGCCTGG AGCACCCTG
 CAGCCAGAAA GCGAGGAAG GTGCTGAGCT ACTGGTGCTT CAGTCCCGGC CACAGCATGC
 ACGAGCTGGT CCGCCAGGGC GTCCGCTCCC TCATCCTTAC CAGCGGCACG CTGGCCCCGG
 TGTCCTCCTT TGCTCTGGAG ATGCAGATCC CTTTCCAGT CTGCCTGGAG AACCCACACA
 TCATCGACAA GCACCAGATC TGGGTGGGGG TCGTCCCCAG AGGCCCCGAT GGAGCCCAGT
 10 TGAGCTCCGC GTTTGACAGA CGGTTTTCGG AGGAGTGCTT ATCCTCCCTG GGAAGGCTC
 TGGGCAACAT CGCCCGCGTG GTGCCCTATG GGCTCCTGAT CTTCTTCCCT TCCTATCCTG
 TCATGGAGAA GAGCCTGGAG TTCTGGCGGG CCCGCGACTT GGCCAGGAAG ATGGAGGCGC
 TGAAGCCGCT GTTTGTGGAG CCCAGGAGCA AAGGCAGCTT CTCCGAGACC ATCAGTGCTT
 ACTATGCAAG GGTGCGGCC CCTGGGTCCA CCGGCGCCAC CTTCTGGCG GTCTGCCGGG
 15 GCAAGGCCAG CGAGGGGCTG GACTTCTCAG ACACGAATGG CCGTGGTGTG ATTGTACGG
 GCCTCCCGTA CCCCCACGC ATGGACCCCC GGTTGTCTT CAAGATGCAG TTCCTGGATG
 AGATGAAGGG CCAGGGTGGG GCTGGGGGCC AGTTCCTCTC TGGGCAGGAG TGGTACCGGC
 AGCAGGCGTC CAGGGCTGTG AACCAGCCCA TCGGGCGAGT GATCCGGCAC CGCCAGGACT
 ACGGAGCTGT CTTCTCTGTG GACCACAGGT TCGCCTTTGC CGACGCAAGA GCCCAACTGC
 20 CCTCTGGGT GCGTCCCCAC GTCAGGGTGT ATGACAACTT TGGCCATGTC ATCCGAGACG
 TGGCCAGTT CTTCCGTGTT GCCGAGCGAA CTATGCCAGC GCCGGCCCCC CGGGCTACAG
 CACCCAGTGT GCGTGGAGAA GATGCTGTCA GCGAGGCCAA GTCGCCCTGGC CCTTCTTCT
 CCACCAGGAA AGCTAAGAGT CTGGACCTGC ATGTCCCCAG CCTGAAGCAG AGGTCCTCAG
 GGTCAACAGC TGCCGGGGAC CCCGAGAGTA GCCTGTGTGT GGAGTATGAG CAGGAGCCAG
 25 TTCCTGCCCC GCAGAGGCC AGGGGGCTGC TGGCCGCCCT GGAGCACAGC GAACAGCGGG
 CGGGGAGCCC TGGCGAGGAG CAGGCCACCA GCTGCTCCAC CCTGTCCCTC CTGTCTGAGA
 AGAGGCCGGC AGAAGAACCG CGAGGAGGGA GGAAGAAGAT CCGGCTGGTC AGCCACCCGG
 AGGAGCCCGT GGCTGGTGCA CAGACGGACA GGGCCAAGCT CTTATGGTG GCCGTGAAGC
 AGGAGTTGAG CCAAGCCAAC TTTGCCACCT TCACCCAGGC CCTGCAGGAC TACAAGGGTT
 30 CCGATGACTT CGCCGCCCTG GCCGCTGTC TCGCCCCCT CTTTGCTGAG GACCCCAAGA
 AGCACAACCT GCTCCAAGGC TTCTACCAGT TTGTGCGGCC CCACCATAAG CAGCAGTTTG
 AGGAGGTCTG TATCCAGCTG ACAGGACGAG GCTGTGGCTA TCGGCCTGAG CACAGCATTC
 CCCGAAGGCA GCGGGCACAG CCGGTCCTGG ACCCACTGG AAGAACGGCG CCGGATCCCA
 AGCTGACCGT GTCCACGGCT GCAGCCACG AGCTGGACCC CCAAGAGCAC CTGAACCAGG

GCAGGCCCCA CCTGTCGCCC AGGCCACCCC CAACAGGAGA CCCTGGCAGC CAACCACAGT
 GGGGGTCTGG AGTGCCGAGA GCAGGAAGC AGGGCCAGCA CGCCGTGAGC GCCTACCTGG
 CTGATGCCCCG CAGGGCCCTG GGGTCCGCGG GCTGTAGCCA ACTCTTGGA GCGCTGACAG
 CCTATAAGCA AGACGACGAC CTCGACAAGG TGCTGGCTGT GTTGCCCGCC CTGACCACTG
 5 CAAAGCCAGA GGACTTCCCC CTGCTGCACA GGTTCAGCAT GTTTGTGCGT CCACACCACA
 AGCAGCGCTT CTCACAGACG TGCACAGACC TGACCGGCCG GCCCTACCCG GGCATGGAGC
 CACCGGGACC CCAGGAGGAG AGGCTTGCCG TGCTCCTGT GCTTACCCAC AGGGCTCCCC
 AACAGGCCC CTCACGGTCC GAGAAGACCG GGAAGACCCA GAGCAAGATC TCCTCCTTCC
 TTAGACAGAG GCCAGCAGGG ACTGTGGGGG CGGGCGGTGA GGATGCAGGT CCCAGCCAGT
 10 CCTCAGGACC TCCCCACGGG CCTGCAGCAT CTGAGTGGGG CCTCTAGGAT GTGCCAGCC
 TGCCACACCG CCTCCAGGAA GCAGAGCGTC ATGCAGGTCT TCTGGCCAGA GCCCCAGTGA
 GTGCCACCG AGGCCCCCAG CACACCCAAC GTGGCTTGAT CACCTGCCTG TCCAGCTCTG
 GTGGGCCAAG AACCCACCCA ACAGAATAGG CCAGCCCATG CCAGCCGGCT TGGCCCGCTG
 CAGGCCTCAG GCAGGCGGGG CCCATGGTTG GTCCCTGCGG TGGGACCGGA TCTGGGCCTG
 15 CCTCTGAGAA GCCCTGAGCT ACCTTGGGGT CTGGGGTGGG TTTCTGGGAA AGTGCTTCCC
 CAGAACTTCC CTGGCTCCTG GCCTGTGAGT GGTGCCACAG GGGCACCCCA GCTGAGCCCC
 TCACCGGGAA GGAGGAGACC CCCGTGGGCA CGTGTCCACT TTTAATCAGG GGACAGGGCT
 CTCTAATAAA GCTGCTGGCA GTGCCC (SEQ ID NO:1) .

The above-exemplified isolated DNA molecule shown in Figure 1A-B and
 20 SEQ ID NO:1 comprise 4946 nucleotides, with an initiating Met at nucleotides 828-
 830 and a "TAG" termination codon at nucleotides 4585-4587. The initiating Met and
 TAG termination codon are underlined.

The present invention also relates to biologically active fragments or mutants
 of SEQ ID NO:1 which encode a mRNA molecule expressing a novel DNA helicase,
 25 NHL. Any such biologically active fragment and/or mutant will encode either a
 protein or protein fragment which at least substantially mimics the biological
 properties of the human NHL protein disclosed herein in Figure 2 and as set forth as
 SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to
 nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-
 30 terminal truncations such that these mutations encode mRNA which express a
 functional NHL protein in a host cell, so as to be useful for screening for agonists
 and/or antagonists of NHL activity.

The isolated nucleic acid molecules of the present invention may include a
 deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary

DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

- 5 The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification.

- 10 The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the NHL protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ ID NO:1 but still encodes the same NHL protein as SEQ ID NO:2. Such synthetic DNAs are intended to be within the scope of the present invention. If it is desired to express such synthetic DNAs in a particular host cell or organism, the codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host, thus leading to higher levels of expression of the NHL protein in the host. In other words, this redundancy in the various codons which code for specific amino acids is within the scope of the present invention. Therefore, this invention is also directed to those DNA sequences which encode RNA comprising alternative codons which code for the eventual translation of the identical amino acid, as shown below:

- 15 A=Ala=Alanine: codons GCA, GCC, GCG, GCU
 C=Cys=Cysteine: codons UGC, UGU
 D=Asp=Aspartic acid: codons GAC, GAU
 E=Glu=Glutamic acid: codons GAA, GAG
 F=Phe=Phenylalanine: codons UUC, UUU
 G=Gly=Glycine: codons GGA, GGC, GGG, GGU
 H=His =Histidine: codons CAC, CAU
 I=Ile =Isoleucine: codons AUA, AUC, AUU
 K=Lys=Lysine: codons AAA, AAG
 L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
 M=Met=Methionine: codon AUG
 N=Asp=Asparagine: codons AAC, AAU
 P=Pro=Proline: codons CCA, CCC, CCG, CCU
 20 30

Q=Gln=Glutamine: codons CAA, CAG

R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

5 V=Val=Valine: codons GUA, GUC, GUG, GUU

W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Therefore, the present invention discloses codon redundancy which may result in differing DNA molecules expressing an identical protein. For purposes of this
10 specification, a sequence bearing one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are mutations either in the DNA sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for
15 glutamine may not cause a change in functionality of the polypeptide.

It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide having properties that are different than those of the naturally occurring peptide. Methods of altering the DNA sequences include but are not limited to site directed mutagenesis. Examples of altered properties include but
20 are not limited to changes in the affinity of an enzyme for a substrate or a receptor for a ligand.

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification. The nucleic acid
25 molecules of the present invention encoding a NHL protein, in whole or in part, can be linked with other DNA molecules, i.e., DNA molecules to which the NHL coding sequence are not naturally linked, to form "recombinant DNA molecules" which encode a respective NHL protein. The novel DNA sequences of the present invention can be inserted into vectors which comprise nucleic acids encoding NHL or a
30 functional equivalent. These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode a NHL protein. It is well within

the purview of the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

Included in the present invention are DNA sequences that hybridize to SEQ ID NO:1 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hours to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography. Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The present invention also relates to a substantially purified form of a human NHL protein which comprises the amino acid sequence (1219 amino acid residues) disclosed in Figure 2 and set forth as SEQ ID NO:2. A preferred aspect of this portion of the present invention is a NHL protein which consists of the amino acid sequence disclosed in Figure 2 and set forth as SEQ ID NO:2, as follows:

MPKIVLNGVT VDFPFQPYKC QQEYMTKVLE CLQQKVNGIL ESPTGTGKTL CLLCTTLAWR
EHLRDGISAR KIAERAQGEL FPDRLSSWG NAAAAAGDPI ACYTDIPKII YASRTHSQLT
QVINELRNTS YRPKVCVLGS REQLCIHPEV KKQESNHLQI HLCRKKVASR SCHFYNNVEE
KSLEQELASP ILDIEDLVKS GSKHRVCPYY LSRNLKQQAD IIFMPYNYLL DAKSRRAHNI
DLKGTVVIFD EAHNVEKMCE ESASFDTLPH DLASGLDVID QVLEEQTCAA QQGEPPHPEFS
ADSPSPGLNM ELEDIAKLKM ILLRLEGAID AVELPGDDSG VTKPGSYIFE LFQAEQITFQ
TKGCILDSDL QIIQHLAGRA GVFTNTAGLQ KLADIIQIVF SVDPSGSPG SPAGLGALQS
YKVHIHPDAG HRTAQRSDA WSTTAARKRG KVLSYWCFSP GHSMHELVRQ GVRSLILTSG

TLAPVSSFAL EMQIPFPVCL ENPHIIDKHQ IWVGVPVPRGP DGAQLSSAFD RRFSEECCLSS
 LGKALGNIR VVPYGLLIFF PSYPVMEKSL EFWRARDLAR KMEALKPLFV EPRSKGSFSE
 TISAYYARVA APGSTGATFL AVCRGKASEG LDFSDTNGRG VIVTGLPYPP RMDPRVVLKM
 QFLDEMKGQG GAGGQFLSGQ EWYRQQASRA VNQAIGRVIR HRQDYGAVFL CDHRFAFADA
 5 RAQLPSWVRP HVRVYDNFGH VIRDV AQFFR VAERTMPAPA PRATAPSVRG EDAVSEAKSP
 GPFFSTRKAK SLDLHVPSLK QRSSGSPAAG DPESLCEVEY EQEPVPARQR PRGLLAALAH
 SEQRAGSPGE EQAHSCSTLS LLSEKRPAEE PRGGRKKIRL VSHPEEPVAG AQTDRAKLFM
 VAVKQELSQA NFATFTQALQ DYKGSDDFAA LAACLGPLFA EDPKKNLLQ GFYQFVRPHH
 KQQFEEVCIQ LTGRGCGYRP EHSIPRRQRA QPVLDPGTGR APDPKLTVST AAAQQQLDPQE
 10 HLNQGRPHLS PRPPPTGDPG SQPQWGSVP RAGKQGQHAV SAYLADARRA LGSAGCSQLL
 AALTAYKQDD DLDKVLAVLA ALTTAKPEDF PLLHRFSMFV RPHHKQRFSSQ TCTDLTGRPY
 PGMEPPGPQE ERLAVPPVLT HRAQQPGPSR SEKTGKTQSK ISSFLRQRPA GTVGAGGEDA
 GPSQSSGPPH GPAASEWGL* (SEQ ID NO:2) .

The present invention also relates to biologically active fragments and/or
 15 mutants of the human NHL protein comprising the amino acid sequence as set forth in
 SEQ ID NO:2, including but not necessarily limited to amino acid substitutions,
 deletions, additions, amino terminal truncations and carboxy-terminal truncations such
 that these mutations provide for proteins or protein fragments of diagnostic,
 therapeutic or prophylactic use and would be useful for screening for agonists and/or
 20 antagonists of NHL function.

Another preferred aspect of the present invention relates to a substantially
 purified, fully processed NHL protein obtained from a recombinant host cell
 containing a DNA expression vector which comprises a nucleotide sequence as set
 forth in SEQ ID NO:1 and expresses the human NHL protein. It is especially
 25 preferred is that the recombinant host cell be a eukaryotic host cell, such as a
 mammalian cell line.

As with many proteins, it is possible to modify many of the amino acids of
 NHL protein and still retain substantially the same biological activity as the wild type
 protein. Thus this invention includes modified NHL polypeptides which have amino
 30 acid deletions, additions, or substitutions but that still retain substantially the same
 biological activity as a respective, corresponding NHL. It is generally accepted that
 single amino acid substitutions do not usually alter the biological activity of a protein
 (see, e.g., *Molecular Biology of the Gene*, Watson *et al.*, 1987, Fourth Ed., The
 Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989,

Science 244:1081-1085). Accordingly, the present invention includes a polypeptide where one amino acid substitution has been made in SEQ ID NO:2 wherein the polypeptide still retains substantially the same biological activity as a corresponding NHL protein. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ ID NO:2 wherein the polypeptide still retains substantially the same biological activity as a corresponding NHL protein. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions.

One skilled in the art would also recognize that polypeptides that are functional equivalents of NHL and have changes from the NHL amino acid sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, (i.e, minimizing the differences in amino acid sequence between NHL and related proteins. Small deletions or insertions are generally in the range of about 1 to 5 amino acids). The effect of such small deletions or insertions on the biological activity of the modified NHL polypeptide can easily be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding NHL and then expressing the DNA recombinantly and assaying the protein produced by such recombinant expression.

The present invention also includes truncated forms of NHL which contain the region comprising the active site of the enzyme. Such truncated proteins are useful in various assays described herein, for crystallization studies, and for structure-activity-relationship studies.

The present invention also relates to isolated nucleic acid molecules which are fusion constructions expressing fusion proteins useful in assays to identify compounds which modulate wild-type NHL activity, as well as generating antibodies against NHL. One aspect of this portion of the invention includes, but is not limited to, glutathione S-transferase (GST)-NHL fusion constructs. Recombinant GST-NHL fusion proteins may be expressed in various expression systems, including *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) using a baculovirus expression vector (pAcG2T, Pharmingen). Another aspect involves NHL fusion constructs linked to various markers, including but not limited to GFP (Green fluorescent protein), the MYC epitope, and GST. Again, any such fusion constructs may be expressed in the cell line of interest and used to screen for modulators of one or more of the NHL proteins disclosed herein.

Any of a variety of procedures may be used to clone NHL. These methods include, but are not limited to, (1) a RACE PCR cloning technique (Frohman, et al., 1988, *Proc. Natl. Acad. Sci. USA* 85: 8998-9002). 5' and/or 3' RACE may be performed to generate a full-length cDNA sequence. This strategy involves using

5 gene-specific oligonucleotide primers for PCR amplification of NHL cDNA. These gene-specific primers are designed through identification of an expressed sequence tag (EST) nucleotide sequence which has been identified by searching any number of publicly available nucleic acid and protein databases; (2) direct functional expression of the NHL cDNA following the construction of a NHL-containing cDNA library in

10 an appropriate expression vector system; (3) screening a NHL-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled degenerate oligonucleotide probe designed from the amino acid sequence of the NHL protein; (4) screening a NHL-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the NHL protein. This partial

15 cDNA is obtained by the specific PCR amplification of NHL DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence known for other kinases which are related to the NHL protein; (5) screening a NHL-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA or oligonucleotide with homology to a mammalian NHL protein. This

20 strategy may also involve using gene-specific oligonucleotide primers for PCR amplification of NHL cDNA identified as an EST as described above; or (6) designing 5' and 3' gene specific oligonucleotides using SEQ ID NO: 1 as a template so that either the full-length cDNA may be generated by known RACE techniques, or a portion of the coding region may be generated by these same known

25 RACE techniques to generate and isolate a portion of the coding region to use as a probe to screen one of numerous types of cDNA and/or genomic libraries in order to isolate a full-length version of the nucleotide sequence encoding NHL.

It is readily apparent to those skilled in the art that other types of libraries, as well as libraries constructed from other cell types or species types, may be useful for

30 isolating a NHL-encoding DNA or a NHL homologue. Other types of libraries include, but are not limited to, cDNA libraries derived from other cells.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have NHL activity. The selection of cells or cell lines for use in preparing a cDNA library to isolate a cDNA encoding

NHL may be done by first measuring cell-associated NHL activity using any known assay available for such a purpose.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for
5 example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Complementary DNA libraries may also be obtained from numerous commercial sources, including but not limited to Clontech Laboratories, Inc. and Stratagene.

It is also readily apparent to those skilled in the art that DNA encoding NHL
10 may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by standard techniques well known in the art. Well known genomic DNA library construction techniques can be found in Sambrook, et al., *supra*. One may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the NHL gene can be isolated, using
15 probes based upon the NHL nucleotide sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou et al., 1994, *Nature Genet.* 6:84-89).

In order to clone a NHL gene by one of the preferred methods, the amino acid sequence or DNA sequence of a NHL or a homologous protein may be necessary. To
20 accomplish this, a respective NHL protein may be purified and the partial amino acid sequence determined by automated sequenators. It is not necessary to determine the entire amino acid sequence, but the linear sequence of two regions of 6 to 8 amino acids can be determined for the PCR amplification of a partial NHL DNA fragment. Once suitable amino acid sequences have been identified, the DNA sequences capable
25 of encoding them are synthesized. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the NHL sequence but others in the set will be capable of hybridizing to NHL DNA even in the presence of DNA
30 oligonucleotides with mismatches. The mismatched DNA oligonucleotides may still sufficiently hybridize to the NHL DNA to permit identification and isolation of NHL encoding DNA. Alternatively, the nucleotide sequence of a region of an expressed sequence may be identified by searching one or more available genomic databases. Gene-specific primers may be used to perform PCR amplification of a cDNA of

interest from either a cDNA library or a population of cDNAs. As noted above, the appropriate nucleotide sequence for use in a PCR-based method may be obtained from SEQ ID NO:1 either for the purpose of isolating overlapping 5' and 3' RACE products for generation of a full-length sequence coding for NHL, or to isolate a
5 portion of the nucleotide sequence coding for NHL for use as a probe to screen one or more cDNA- or genomic-based libraries to isolate a full-length sequence encoding NHL or NHL-like proteins.

This invention also includes vectors containing a NHL gene, host cells containing the vectors, and methods of making substantially pure NHL protein
10 comprising the steps of introducing the NHL gene into a host cell, and cultivating the host cell under appropriate conditions such that NHL is produced. The NHL so produced may be harvested from the host cells in conventional ways. Therefore, the present invention also relates to methods of expressing the NHL protein and biological equivalents disclosed herein, assays employing these gene products,
15 recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these assays which act as agonists or antagonists of NHL activity.

The cloned NHL cDNA obtained through the methods described above may be recombinantly expressed by molecular cloning into an expression vector (such as
20 pcDNA3.neo, pcDNA3.1, pCR2.1, pBlueBacHis2 or pLITMUS28) containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant NHL. Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned DNA and the translation of their mRNAs in an appropriate
25 host. Such vectors can be used to express eukaryotic DNA in a variety of hosts such as bacteria, blue green algae, plant cells, insect cells and animal cells. Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a
30 limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. To determine the NHL cDNA sequence(s) that yields optimal levels of NHL, cDNA molecules including but not

limited to the following can be constructed: a cDNA fragment containing the full-length open reading frame for NHL as well as various constructs containing portions of the cDNA encoding only specific domains of the protein or rearranged domains of the protein. All constructs can be designed to contain none, all or portions of the 5' and/or 3' untranslated region of a NHL cDNA. The expression levels and activity of NHL can be determined following the introduction, both singly and in combination, of these constructs into appropriate host cells. Following determination of the NHL cDNA cassette yielding optimal expression in transient assays, this NHL cDNA construct is transferred to a variety of expression vectors (including recombinant viruses), including but not limited to those for mammalian cells, plant cells, insect cells, oocytes, bacteria, and yeast cells. Techniques for such manipulations can be found described in Sambrook, et al., *supra*, are well known and available to the artisan of ordinary skill in the art. Therefore, another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding the NHL protein. An expression vector containing DNA encoding a NHL-like protein may be used for expression of NHL in a recombinant host cell. Such recombinant host cells can be cultured under suitable conditions to produce NHL or a biologically equivalent form. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Commercially available mammalian expression vectors which may be suitable for recombinant NHL expression, include but are not limited to, pcDNA3.neo (Invitrogen), pcDNA3.1 (Invitrogen), pCI-neo (Promega), pLITMUS28, pLITMUS29, pLITMUS38 and pLITMUS39 (New England Biolabs), pcDNAI, pcDNAIamp (Invitrogen), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and IZD35 (ATCC 37565). Also, a variety of bacterial expression vectors may be used to express recombinant NHL in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant NHL expression include, but are not limited to pCR2.1 (Invitrogen), pET11a (Novagen), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia). In addition, a variety of fungal cell expression vectors may be used to express recombinant NHL in fungal cells. Commercially available fungal cell expression vectors which may be suitable for

recombinant NHL expression include but are not limited to pYES2 (Invitrogen) and *Pichia* expression vector (Invitrogen). Also, a variety of insect cell expression vectors may be used to express recombinant protein in insect cells. Commercially available insect cell expression vectors which may be suitable for recombinant expression of NHL include but are not limited to pBlueBacIII and pBlueBacHis2 (Invitrogen), and pAcG2T (Pharminogen).

Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of bovine, porcine, monkey and rodent origin; and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. For instance, one insect expression system utilizes *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) in tandem with a baculovirus expression vector (pAcG2T, Pharminogen). Also, mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), Saos-2 (ATCC HTB-85), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171) and CPAE (ATCC CCL 209).

As disclosed in Example section 1, a 115 kb BAC clone (from Genome Systems) was subcloned and subjected to restriction and sequence analysis. Four genes at chromosome location 20q13.3 were identified, including M68/DcR3, NHL, SCLIP and ARP (Figure 5A). The nucleotide sequence of this BAC clone, hbm168, is presented as follows:

25	TGAAGAGCTT TGACCAAGAG GCTGTGACGA GGCCCTACGA GGA CTCTGGC TCTCCTCCTG	60
	CTAAGCACAC CCAGGCAGGT GTCCTGGCAG ATGAGGACCA CATGCAGAGC CTCGGCCAGC	120
	CCACCAATGC CCGGATATGC AAGTGAGCCC AGCCTGGACC CCCC GGCGAG GCCCAGCAGC	180
	ACCAGCCCAG GCCCGAAAAC CTTAAGAAAT GACCAGTGTC TGCTGCTTTA AGCCACCAAG	240
	CTCTGCGGTG GTTTGTTAGG CTGCAAGCAT GGCTAATTCA GAAACTGCCA GAAACAAGCA	300
30	CTGCTGTCCC CAGCCTGGGA CACACAGCAC CGCCTCTGCG TGGGGAGAGG GCACAGGCTA	360
	AGGGCACAAA TGCCATCCCA GACCCGGCTC TTGTGTGTGG AAGGGGCCAC TGTGCCATGA	420
	GGCAGAGGAA ACCTTGGCAG GACCTTATGC CACAGCAATT TAAAAGAGAA GAAACAGGCT	480
	GGGCGTGGTG GCTCATGCCT ATAATCCCAG CACTTTGGGA GGCCAAGGTG GTGGATCACT	540
	TGAGGTCAGG AGTTCAAGAC CAGCCTGGCC AATATGGTGA AACCTGTCT CTACGAAAAA	600

	TACAAAATTT	AGGCAGGCGT	GGTGGCGGGT	GCCTGTAATC	CCTGCTATTC	AGGAGGCTGA	660
	GGCAAGAGAT	TTACTTGAAC	CCAGGAGGTG	GAGGCTGCTG	CAGTGAGCTG	AGATCATGCC	720
	ACTGCACTCC	AGCCTGTGTG	ACGGAGTGAG	ACTTGGTCTC	AAAAAAAAAA	AAGGAAACAC	780
	ATCTGACTAG	TGTGATCTCG	CAAGGAACAT	TCCAGACACA	GTGGAGCTAG	AAGGTTCTTC	840
5	TCCAAACAAG	GAATCCCCAG	GGGATCAAAT	TGTTTTGCAT	CGGCCAGACA	TGGTGGCTCA	900
	AGCCTGTAAC	CCCAGTGCTT	CGGGAGGCTG	AGGTGGGAGG	ACTGCTTGAG	TCCAGGAGTT	960
	CAAGACTAGC	TTGGGCAACA	CAGTGAGAGC	CCATTAGCCA	GGCGTGGTGG	CACATGCCTG	1020
	CAGTCCCAGC	ACTGTACTAA	AAATCTACAC	GGGGCCGGGC	ATGGTGGCAC	ATGCCTGTAG	1080
	AGTCCCAGCT	ACTCAGGAGG	CTGAGGCAGG	ACGATTCCCT	GAACCCAGGA	GGTCACGGCT	1140
10	GCCATGAGCC	GTGACTGTGC	CACTGCACTC	CAGTCTGTGC	AACAGAACGA	GACTCTGTTT	1200
	CGAAAAACAA	AAAATCATTT	CATGTCTCCA	GTTTCTCCAC	TGGCAAAAGA	CTCTGTCAAG	1260
	GTAAAAATG	GTTCTGACCC	ACAGAAATCT	AAGAAAGGAA	AAAATATAAA	AAATAGAAAA	1320
	TTTAAAAAAG	AGATGGTCTC	AGAATAAAGA	CCAACCTGGG	CTATGGTTGT	CACTCTTCCC	1380
	TCACACCTTA	GAAAGCTTTC	TGGCCGCATC	TGGCCAAAGG	GCCACCCTGC	CCCATCTTGG	1440
15	ATCAGTGAGG	TGCCTTCGAA	CAAGCCACCT	GCCCTGGAGC	CCGTCTGTGC	TTGTCTGCCA	1500
	CCGCACGCTC	AGTAGGGGAG	GGGAAGTCGC	TAGGTTTTAG	TTCACCAGTC	TCTGGATCAA	1560
	GACGTGCCAT	AACCAAGAAG	CCCCAGCCAC	ACCCAGACCC	GATGTGGCCA	CAAGGGGTGA	1620
	GCTGGGAAGG	CCCAGGAAAA	GGCGGGAGGC	GGACGAATGG	AAATGTCATT	CTGTGGCCAC	1680
	AGAAATGATC	TCAACGTTTT	GTAACCTCCT	ACCAAGAGGC	AGTCTTAGCT	CTGCCCTTGA	1740
20	ACCAGCACTT	GGTGATGTCG	CTTGCGTCAA	TCAAGGCAAC	AGAAGTGAGC	AGGAGGCCCA	1800
	CTTTCCTCTG	CAACTGTGGG	CTTACGGGGC	AAAGAAGTCC	AGGCCTCCAG	GTGGAGGATC	1860
	ACAGACCGGG	CAAAGCAGAG	GAGAGCCACC	CAGCCGAGCC	TACCTGTGCC	TCAGACTGCC	1920
	TCCCTCCAGA	GACCCCTGTG	GCCAAGGCCA	CCCAGACCAG	CAGGTCCTTG	CCAAGCTGTC	1980
	AGCTGACGAC	AGGGGTGGT	GAGGCCGGCC	CAGACCAGCA	GAACCACGAA	CCAACCAACA	2040
25	GAATTAAAAA	TAATAACAAC	TATGTCTTGT	CTTAAGCCAC	TAAGTTTGG	ATGGTTTCTT	2100
	TCTTTCTTTT	TCTTTTTTTT	TTTCGGAGAC	GCAGTCTCAC	TCTGTGCCC	AGGCTGGAGT	2160
	GCAGTGGCGC	AATCTTGGCT	CACTGCAAGC	TCTGCCCCC	GGATTCACGC	CATTCCCCTG	2220
	CCTCAGCCTC	CTGAGTAACT	GGGACTACAG	GTGCCTGCCA	TTGGGTGTTT	TCTTAAACAG	2280
	CAAAAGAAAA	CTGACACAAT	CATAAACAGA	GCAAGCAAGA	GAACCTGGCA	ATTATTTCTT	2340
30	CTCTACTTCT	CACTGTTCCT	CAAAGAGTTA	ACTCAAGCAT	AAGATGTGAG	CAAATTCTTT	2400
	TAACATCCTA	GAAAAAAGC	TCCTACTCAG	TGTTCATAAA	GCAAAGCTAA	CCTACAGGAG	2460
	CCACCTTCCA	CAGTGACCAC	AGGAAACCAA	GACAGCAAGT	GGGACACCAG	CCTCCAGGGC	2520
	ACTGCGCCAG	CCGTGCGCCT	GTGTCTGCCA	CTGCCCTGGT	CCGTCACTGC	CACCAGCCGG	2580
	CAAGACACCC	ACAGAGGAGA	GCTCTAAGCC	ACAACGTGTG	ACGAAGACAA	CTGTGCAGGA	2640

	TTTTATTACT	ACAACATTTT	TGTTTTCTTT	TTTTTTTTTT	TTTGAGACTG	AGTCTCGCTC	2700
	TGTCACCCAG	GCTGGAGTGC	AGTGGCACAA	TCTCGGCTCA	CTGTAACCTC	CATCTCCCTG	2760
	GTTCAAGCAA	TTCTCCTGCT	GCAGCCTCCC	AACTGGATTA	CAGGCGCCCG	CCACCACGCC	2820
	TGGCTAATTT	TTGTACTTTT	AGTAGAGATG	GGGTTTCACC	ATGTTGGCCA	GACTGGTCTC	2880
5	AAATTCTGA	CAAGTGATCC	ACCCACCCTG	GCCTCCCAAA	GTGCTGGGAT	TACAGGTGTG	2940
	AGCCACTGCG	CCTGGCCCAT	TTTTGTTTAT	CAATAAAAAT	GTAATTAAATG	TTGAACTCTC	3000
	CACATTTCAA	ATGGGTAACT	CCAGTGTCTT	TGATGCTCCT	GCGACATGTT	CGTGAGACTT	3060
	CTCTTGGGTG	TGAGAGTCTA	GCATGTGGGT	GGTCTGGACA	GGAGGGGGAG	GGAAGAGTGC	3120
	AGAGCCGGGC	AGGGTAAAGA	GACCCCTAG	GATGTGAAGG	CCGCCCTGCA	TTTGTGAGAC	3180
10	TGGGCAACAC	CCACTCCATC	AGATGGACCC	TGGTATGGGC	GGCAAGCCAC	CTAGGTGCCG	3240
	AGGCAAGAGA	CCGAGGGCAC	GAGCTGTTCC	GGTGAATAA	AATGCATAAA	ATAAGAATAG	3300
	TTATACTAGA	TATAGATCAT	AAATATGATT	ATATATGAAT	ATCATTCATC	ATTAGTTTGT	3360
	AGCAATTACT	CTTTATTCCA	ATATTATAAT	AATCCTTGCC	TAAGCATAAC	CTAGGAAAAA	3420
	CTAGGAAATC	ATAACCTAGG	AAAACTAGG	CCATACAGAG	ATAGGAGCTG	AGGGGACATA	3480
15	GTGAGAACTG	ACCAGAAGAC	AAGAGTGCAG	GCCTTCTGTT	ATGCCTGGAC	AGGGCCACCA	3540
	GAGGGCTCCT	TGGTCTAGCG	GTAACGCCAG	CATCTGGGAA	GACGCCCGTT	GCCAAGTGGA	3600
	CCGTGGTCTA	GCGGTAGCCT	CAGTGTCAAG	GAAAAACACC	CGCTACTTAG	CAAACCAGGA	3660
	AAGAGAGTCT	CCCTTTCCCC	GGGGGAGTTT	AGAGAAGACT	CTACTCCTCC	ACCTCTTGCG	3720
	GAGGGCCTGA	CATCAGTCAG	GCCCGCCCGC	AGTTATCCGG	AGGCCTAACC	GTCTCCCTGT	3780
20	GATGCTGTGC	TTCAGTGGTC	ACGCTCCTAG	TCCGCCCTTCA	TGTTCCATCC	TGTGCACCTG	3840
	GCTCTGCCTT	CTAGATAGCA	GCAGCAAATT	AGTGAAAGTA	CTGAAAGTCT	CTGATAAGCA	3900
	GAAATAATGG	CGTAAGCGGT	CTCTCTCTCT	CTCTCCTCTC	TCTCTGCCTC	AGCTGCCAGG	3960
	AAGGGAAGGG	CCCCCTGGCC	AGTGGGCACG	TGACCCACAT	GACCTTACCT	ATCACTGGAC	4020
	ATGGTTTACA	CTCCTTACCC	TGCCGCTTTG	TCTTGTATCC	AATAAATAGC	GCAACCTGGC	4080
25	ATTCGGGGCC	GCTACCAGTC	TCCGCGTCTT	GGTGGTAGTG	GTCCCCCAGG	CCCAGCTGTC	4140
	TTTTTCTTTT	ATCTTTGTCT	TGTGTCTTTA	TTTCTACACT	CTCTCATCTC	CGCATACGAG	4200
	GAGAAAACCC	ACCAACCCTG	TGGGGCTGGT	CCCTACACCC	TGGCTTTGTA	GACTGGAGCC	4260
	TAGGCACGAC	TCAGCTGCTG	TAGTGAATTG	CGATCCTCCA	AACCCAGCAA	GGCACCTGCA	4320
	GGACATCTGG	CCCAGTCTCC	TCGTTGAGCC	AGTTCACGAA	AAAGAGACTT	TTCTGAGTGA	4380
30	CATGCTAATG	GGCAATATGA	GGACTAAATG	GGATGGTCTC	CAACTTGGAC	AAACCAACAG	4440
	TAAAAGCCAC	TTTGCGGGGA	AAGAACTTTT	TCCTTTTTTTC	TTTTTTTTTGA	GACAGGATCT	4500
	CACCCTGTCA	CCCAGGCTGC	AGTGCAGTGG	CATGACCTTG	GCTCACTGCA	GCCTCAACCT	4560
	CTCTCAGGCT	CAAGCAATCC	TCCCGCCTCA	ACCTCCCATG	CAGCTGGGAC	CATAGGTGCA	4620
	TGCCACCACA	CCCAAATAAT	TTTTATATTT	TTTGTAGAGA	CGAGGTTTCA	CTATGTTGCT	4680

	CGGGCTGGTC	TCAACTCCTG	GGCTCAAGCA	ACCCTCCCAC	CTCAGCCTCC	CAAAGTGCTC	4740
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	AATATTAGAA	GAGATGAAAA	TTCATCAACA	TGGAAAGACA	AAGATCATTA	ACTAAAGCCA	4860
	AACCAGAATG	GAAGCTGTGT	GTACAGTGGG	GTCTCATGCT	GGGAACGCGA	GGGGCACGTG	4920
5	CAGGGCTCCA	CGGTGTGGCG	ACGCCCCATG	CTCCCTTTGT	GGGGGTTTCAT	CCAGCGGAAC	4980
	ATGAGGACCT	GGGGTGCTTT	TCAACATGTA	CGTGAGTTTA	ATAATAAAAA	GGTTTAAGGA	5040
	AAGAAAAATT	CATATGTTTC	TATATAAACA	GAACATCTGG	AAAGATCTAT	TCTAAGGTGT	5100
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	GCACTTTGGG	AGGCTGAGGC	AGGCAGATCA	CCTAAGGTCA	GGAGTTTGAG	ACCAGCCTGG	5400
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15	CAGAGGTTGT	AGTGAGCCGA	GATCATGCCA	CTGCACTCCA	GCCTAGGCAA	CAAGAGCGAG	5580
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	CCCTGCCTAC	AGGACCTGA	GAGCTAGGGG	AAGGCGTTAT	CCTGAACTGT	GTCCCCCGTA	6000
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25	GTGGTTTTTT	TTTTTTTGGA	GACTGGGTCT	CACTCTATCA	CTCAGGTTGG	AGTACAGTGG	6180
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	CTCCTGAGTA	GCTGGGACTA	CAGGTGCTTA	CCACCGCACC	CAGCTGGTGG	TGCATTGTGT	6300
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	CATCCCGGCA	GCCGCGGCCA	CGGTGTCACG	GCTCGGATGA	GATGACTCGG	ACACCACCCC	6900
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	CGGCCGCCCC	TCCCCCGGCC	CTCCCCTCCC	CCCGCCGTAA	CGTCCTGACG	CTCCGCAGGG	16020
20	ACCCCTGACT	GGACGGCGGC	GCGTGAGCGG	AGCGAGAGGC	CTCGCCCGCG	GGGGGCCGCG	16080
	GGCTCGCCGG	CGCCGCTTAC	CTGGGGCCCG	GCCGGGCCCTG	CTTAGGCACC	CGGCGGGGGC	16140
	GGCGGCGTCG	GGAGCTGCGG	CGGCGGCGGG	CGGCGGCGGC	GGCCGCGGGC	TTCGCTCCTT	16200
	GTTGGGGATT	CGGCGGCGGC	GGCGGCGCGG	GCGCGCGCTT	CCTAGTGACG	CAGGCGGCGG	16260
	GGCCGCGCAC	GCACGGGGCT	GGGAGGGCCG	GACACTTATT	TGGCGCTCGC	GGAGGAGGAA	16320
25	GGCGGGGCCG	TGAAATAAGG	CCCACGCGGC	CCCGGGGCGC	GTGCGCGGAC	CGACACTGTC	16380
	AGCTCCTAAC	GCCGCAGGTT	CCTCCTGGTC	CCCAGGCCCC	CCGGTCGGGC	GTTCCTGCTC	16440
	CCGCGCGGGC	GGCCGGGCCG	AGGGACGATG	GTCAGTGAGC	GGACGGCGCC	AGGGAGCAGT	16500
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30	GCTGTGGAGG	AGGACGCTGT	AGGGTGCGCG	GAATCAGCG	GAACATGCCA	GAGGCTCAGC	16680
	CAGCCACGGC	GCTCCCAGCG	TGGAGGGCGA	GGGGCATCCG	GGAGCGGCCG	GGAGGGCTCG	16740
	GTCACCCCTC	AAGCTGTCAC	CCCAGTCCCA	CAACCAGCAC	CCCGATCCTA	TCGCAGTCCC	16800
	ACAGCCGACA	CCCCGATCCC	ACCCCTGCCC	AACAGCCGGC	ACCCACCCCA	ATCCCATAGC	16860
	TAACACCCCG	GTCCACCCGC	TGTCCACCGG	CCGGCACCCC	GATCCACCCC	CAGTCCCGCA	16920

	GCTGGCACCC	CGATCCCACC	CCAGCCCAAC	AGCTGGCACC	CACCCCGATC	CCACCGCTGT	16980
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	GGCACTCACC	CCGATCGCAT	AGCATAGCTG	ATACCCCGAT	CCCACCCAG	TCCCATAGCC	17100
	AGCACCCCGA	TCCCACCCCA	GTCCCATAGC	CAGCACCTCG	ATCCCATAGA	TGACACCCCG	17160
5	ATCACGCCCC	AGTCCTATAG	CCCGCACCCC	GATCCCACCC	GAGTCCCGCA	GCCGGCACCC	17220
	CATCCCACCC	ATGTCCCACA	GTCCGGCACCC	CGATCCCCT	CGGATCCGGC	AGCCAGCTTG	17280
	GATCCTGTGG	CCCTCCTCCA	GCCCCAGGG	CTCATTTATA	TGTTTTATTG	GCAGAGGCTG	17340
	GGGCTGGCTC	TGTTGGCCTC	TGTGCTGGGT	TTCTTCCTCT	GCACCGCAGG	ACTGGCTCTC	17400
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	TCCCGGAACG	GGAGGTTCCT	AAGCCACTGC	ACTTCGGAGG	GGCTGTAATT	AGAGTTGCAC	17640
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15	AGGTGGGAGG	ATCACCTGTG	GCCAGGAGTT	CAAGACCAGC	CTGGGCAACA	GAGCCTGTCT	17820
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	GAGAACTCC	ATCTAAAAAA	AAAGAAAAAT	CACCTCCAAG	ATAACTTAGC	TTTCTTCTGC	18180
	TGGCATAACA	AATTATCTCA	AACTTAGTCG	CTTAAAAATG	CAAATTTAGG	CTGAGTGCGG	18240
	AGGCTCACGC	CCATAATCCT	AGCACTTTGG	GAGGCCAAGG	CAGGATTGCT	TGAGGCCAGG	18300
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25	GGTGGCTCAC	GCCTATAATC	CCAGCACTTT	GTGAGGCCAA	GGCGGGCAGA	TCACGAGGTC	18420
	AGGAGATAGA	GACCATCCTG	GCTAACACTG	TGAAACCCCC	TCTCTACTAA	AAATACAAAA	18480
	AATTAGCCTG	GCGTGGTGGC	AGGCGCCTGT	AGTCCCAGCT	ACTCGGGAGG	CTGAGGCAGG	18540
	AGAATGGCGT	GAACCCAGGA	AGCGGAGCTT	GCAGTGAGCC	GAGATGGCGC	CACTGCACTC	18600
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	GAACAATCTG	ATGAGGAAGA	AGATAGAAAC	AGCTTAAACA	ATAGTCTCCC	GGCCGGGGGC	20880
	AGTGGCTCAC	GCCTGTAATC	TGAGCACTTT	GGGAGGCCGA	GGCGGTGGA	TCACAAGGTC	20940
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	TAGCACCCAT	GTCGTGGAAT	CACAATGTTG	TCGATAGCTC	TGGGGTCTTG	TACAAAATGC	22320
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	GTGAATGGGG	TGAGTGGGGT	GAGTCCACAG	AACAGTGGGG	TGCAGCCCCA	GGGGTCTCGT	28440
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	ATATGTAGGC	CCTGCCCTTT	CCTCCTGAGC	GGCGGGCCAC	AGAGTCTCTGA	ACAACAGGAA	28560
25	GCCCCTGAGG	AGGGCTCCGC	CCTGAGGGAG	GGCAGGGGAG	CCCCCGCCAG	CCCCACCCAC	28620
	AGCAGCGGGC	CCTGCCACCC	CCCACCCTGA	CACCTCACCC	CTTGATTCC	AGAGAGGAAA	28680
	GTGGGCTTGT	GTGTAGTTTA	CATGCTCATA	TCTTAAATC	ACCGTTGTCA	ATAGAACAAT	28740
	TCATAATAAT	GATGATAAAA	TAAGATTTAT	AACCAGCTTC	AGTCTGGAGA	TACACACAGA	28800
	GCAGATCTTC	ACTCCAGAC	AGGGAGCCCG	CAGCTGCCCC	CGACCCACAC	GGTGCAGGAC	28860
30	ACACACAGAC	AGTTCAACCA	TGTCTTAAAC	ACACAGGTGT	TTATTTAATT	GTTCATTTGA	28920
	TTGAATTTTT	AAGTTCACTT	TACTACGTGG	ATGAGATGGG	TGCATATTAC	AGTAGGCTTT	28980
	CGCTATGAGC	GCTGCCACCA	TGAGGAATAT	CCCAGCCCTC	AGTCTGCTT	CCCTTTCTGA	29040
	GTCCCACAAA	AGCCAGATGT	GGACAGCCTT	GGGTTCCCAT	CCCAGCTGGC	TGCTCCTTCT	29100
	GGGGCTGTCT	TGGTGGGGAG	AGGGAGATGG	GGCAGTGGGT	CCCTGCTGAC	CCCTGAGCCC	29160

	TGCAGGGGTC	AGGATCCTCC	CGTGGTCCCT	GGGTGTGGCT	CTGGAAGACA	CTGGCAGTGC	29220
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	TCAGCCGCCC	CCTTCACACT	TTACAGCAAG	GGGCCAGGCA	GCAGCTTTGG	GATGGGGCTT	29340
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5	CAGCTGACCG	TCCTCCAAGG	CCAGCACTGG	GCGTCCAAGG	GAAAGAAGGA	ACTCAGCCCA	29460
	GAGGGTGTGG	GCAGGAGAGG	CCTGGAGTCA	GGCCTCCACC	CACAGCCCCC	TCTGGGTGCC	29520
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	ATCTGTGGGC	AGCGGTCCCA	CAAAATCCCC	CTTGGGGCTC	CCTGAGGAGG	ACTTGTGGCT	29640
	GCCGCTTCCA	CCAGGGCAGA	GGGCACAGGA	GGGGCCAGCA	CTCCAAAGGG	CTCTAGGGTG	29700
10	GGTCTTTCAA	GGACATCTGC	AAAGCCCTGG	TGGGGAGGGG	CCTGGGCCAG	AGGCTCTTTG	29760
	GAACTCTTGC	ACTTCTGAGT	GGGGGACTGT	CCATGCTGCC	CACAACCTCT	AGACCATGCA	29820
	GCCTGCTCAT	GGGTCCCTGG	CAGAGAATGC	CCACTCCCCA	GCAGACTCAG	GGCAGGCCCC	29880
	CAACTGCAGG	CTTCCAGGAA	GGCCAGGGT	GTCCACCTCA	CGCCAGGTGG	TCTCAGAGGA	29940
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	CCTCACGGGG	AGCCCCTTCC	CCCTGGAAAG	ACAGCAGGTA	CTGTAGCCTC	GCCTGCTGGC	30120
	CAGGGGCGCC	GGCTCAGAGG	ACCTGCCCTG	ACCTGCACGT	GCTGACCAGA	CAGCCCAGCG	30180
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	AGGGCGGCTG	AGTGCGGAAG	AGAAGCATGA	AGCTGGGGGC	GGGGGTGGGG	GAGGAGGAAC	30420
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	CCCCGCCAG	TGCCCCGCCC	CCTGCCTGCT	GCTAGCCCTG	CCCCCGCCCC	GGCCCTTGCC	31080
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	GGTCGGGGCA	GGGCCAGGGG	GTCGCGATCC	GCCGCCCCCG	CCCCCGTCCC	TGCCTCGCGC	31920
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	TTGCAGGCCC	TGTCCCCGTG	GGCCGCCTCC	GTGAAACTC	CTGGGGGGTG	GGGGATGGAG	33120
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25	TCATGGTGCC	AGGAGCACAG	CATGGCAGGA	GCCACCAGCC	GATTGTCCTT	TCCCTGGGCA	40860
	GGAAACTCAG	AAATGTGGCT	ACCACAGTCA	GGCTGCTTGA	CGTGCGGTGA	GCACTCATCT	40920
	CTTAGCAGGC	AAGCGGCCAA	GCACCTTTCC	TGAAATATTG	AGGCCTCAGA	ACAAGCCCCA	40980
	GGAGAGGTGC	CAGCACCGTC	ATCTCTACCC	AGATAAGGAG	ACCCAGGTCC	TGAGAGGTTA	41040
	GGCAGCTCGG	ACAACACCAC	ACAGCTGGAG	GAGGTACAGAC	TCTGGGTTGC	AGAAGGAGAA	41100
30	TGTGAGCAGA	GGCCACAAAA	GAGCGAGGAG	CCAGTGCCCA	GATGCCGAGA	TGCCCTCGCC	41160
	CTCCCAGCTC	AGCCCCAGGA	ACCGAGCCCA	TGGGGAGGGA	CCGTCAGGGA	AAGGCTGTCA	41220
	GGAAGGGCAG	GAGGCGGCCC	TGGAGAGGAC	GGCGCTGCCC	TCAGGGGCAG	GAGGGGAGTC	41280
	CCCTCCGCTG	AGAGCCCCCC	CACCCCCAGT	ATCCCCGGGG	GTGTCCAGGA	GGAGGCGGAG	41340
	GGAGGAAGCG	CAGATGGACA	GGACTCCCAG	ATAGGGTGGG	GAGGTGTGGC	CGGTGACACA	41400

	CACGGTCCCC	TCCTGGCAGG	TGCTGAAGTC	ACCTGGAGCC	TCCAAGCCCG	TGGGGCCTGA	41460
	GGGGCGGGGT	CAGGTCGGGC	ACGCGTGGGT	GGGCGGAGTT	CTGCGCCCCG	GGCCAAGGCG	41520
	CCCAGTTGA	ACCAGTCAGC	TCGGGAGAGG	GACCGCGGCG	ACCTGTCCCG	GGGGCGTAAG	41580
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5	TTCGTCTGT	CTCCCAAAC	GTCGAGTGAA	AAACGAAGCG	GGTTCTGCGG	CCTCGCGGCG	41700
	GAGCAGAGCG	TTTCGGGAAG	GGCGGGCCCA	GCGTCTCTCG	GCCCAGAGTC	GCCCAGGAGC	41760
	TCCCCTGCGT	CCAGAATCCG	CCCCCGCCCC	GGGCCTGCGC	CCGCCCCCTC	GCCTGAGCTC	41820
	CGCGCGGGAC	GGGCCGGGAG	GCCGGGGTGG	GCGCTACCTT	CGAAGGCGGT	GGGTCCGCCC	41880
	CGCGGGAGGT	GGAGGGGCGG	GAGGGGCGGA	GCCCTCTGGT	CTCCGGAGGG	TTTGGGGATC	41940
10	GCAGTCGCCC	CTCCCCATC	CAGACCCCGC	GGCGCAAAGG	GCAGTGGCTT	TTCTGGCCAG	42000
	AGCAGGTGGC	GCGGGCGTCG	CAAAGGGTGG	TCCCCGAGGC	CGCAGCGGTG	TGGGGGGAGG	42060
	GCGCGGTCCC	CCTCACTCCG	GGCTCCGCCG	TGTCTGGCCC	GCCCCCTTCC	TTCAGCGCCC	42120
	CCTCCAGCCC	CTGTGCTGCA	CTGGCGCGGG	GAGCGCCGGG	TTCCCGGCTG	GGGCTTTGGC	42180
	AGAGGGTCCC	ACCTCTTCCC	CGCTCCCCCA	CGAAGGCTCT	GGCGGACCCA	GATCTCGGGT	42240
15	CGCCGGACGC	CCCAGGGACC	CCGCCCCGAC	ATCGCGAGCG	CGCCCACCCG	GTCGCGAGCC	42300
	CACGCCCGGG	TCTGGGAGCC	ACCCTGCGGC	AGTCGCGCCC	TGCGTGGCAC	GCTGCTCCCC	42360
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	CCCAGGCTTG	CAACGCGCAG	GGTAGGAGAA	GGGAAATTGG	CGTCCGCTGC	CGGCCGCTGC	42540
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	TGGAGAGGCG	CAAGTGGCGG	CCGGAGCTGC	AGACGGCTGG	TGCTGCAGTG	CCGGGGAGGG	42720
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25	GGGAGGGTTG	GGGGAAGGAG	AGAGAGAGAG	AGAGAGACTG	CGGGGGCGGG	GGAAGGAGGG	42900
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	CCACAGGGGT	GGGCGCTGGC	CTGGAGGCCT	CCAAGGGGCA	TCTCCTGTGA	GCCCAGGGGA	43320
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	GAGTAATGAC	AGCATGCTGG	CAGGCACACA	ATAGCTGTAC	TGACTGTTTG	CCCCAATATA	45000
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	CACCTCAGCC	TGGGCAACAA	TATCAAGACT	CCACCTCAA	AAAAAAAATG	TTTTTCATAA	45420
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	G	CC	AGG	CCG	TTT	AC	CTG	C	AG	AT	CG	AA	TCT	GT	AC	AG	G	GGC	AG	CC	AC	GAG	TT	65460
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	GCTGCTGAGG	GGCCTGGGCT	GGCGAGGGCC	TGGGTGGGGA	GTGCCTGGGC	CGCCCCTGCC	81480
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	CAGGACTACA	AGGGTTCCGA	TGACTTCGCC	GCCCTGGCCG	CCTGTCTCGG	CCCCCTCTTT	82080
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5	AGTGTGGGCC	AGAGTCCTGG	GCTGCTTGGG	GTGGGCATCC	TCGGGCCCTG	CTTGGCCCCG	82500
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 CAAATTTTTT TCCTGCCCCCT CTCTCATCTC CTTTGGGAG TACCACTGCA TGTATGTTGG 114780
 TGTGCGTTCT CTA (SEQ ID NO:3) 114793.

5 The present invention also relates to a portion of SEQ ID NO:3 which comprises 5' regulatory regions, exons, introns and 3' non-translated regions which comprise the human NHL gene of the present invention. Such regulatory sequence may be found within the various regions of this 115 kb fragment. The 5' portion of SEQ ID NO:1 begins at nucleotide 47095 of SEQ ID NO:3, the initiating ATG of human NHL is from nucleotide 48687-48689
 10 of SEQ ID NO:3, the termination 'TAG' codon is from nucleotide 84855-84857, while the 3' terminus of SEQ ID NO:1 as disclosed herein (GCAGTGCCC) corresponds to nucleotides 85308-85316. To this end, one preferred aspect of the invention is an isolated genomic fragment or fragments which comprise from about nucleotide 470000 to about nucleotide 85500 of SEQ ID NO:3), which comprises the portion of the genomic clone encoding the
 15 mRNA transcript responsible for human NHL (see Figure 5A-B). The genomic sequence encoding NHL contains 35 exons (Figure 5A). An especially preferred aspect of the invention is a human genomic fragment or fragments which comprise from about nucleotide 47095 to about nucleotide 85316 of SEQ ID NO:3. As noted in regard to SEQ ID NO:1, the present invention also relates to DNA vectors and recombinant hosts which comprise at least
 20 a portion of SEQ ID NO:3. Portions of the 115 kb genomic fragment may be housed in multiple vector/hosts so as to optimize handling of the DNA sequences within SEQ ID NO:3. Therefore, the present invention relates to the isolated genomic sequence which set forth as SEQ ID NO:3, a region of SEQ ID NO:3 which contains the coding and non-coding region of human NHL, as well as *cis*-acting sequences within SEQ ID NO:3 which effect regulation of
 25 transcription of one or more of the genes localized within this 115 kb human genomic fragment, including regulatory regions effecting levels of NHL, M68/DcR3, SCLIP and ARP. As noted above, this region of chromosome 20 (20q13.3) is associated with tumor growth. Therefore, an aspect of this invention also comprises, as one example, the use of one or more regulatory regions of this 115 kb genomic sequence as a target to antagonize the effect of a
 30 transcriptional factor(s) which normally upregulate expression of a gene which has a caustic role in tumor growth. Alternatively, compounds may be selected which interacts with a specific *cis*-acting sequence to upregulate a gene within this region, where upregulation results in a decrease in tumor growth.

The present invention is also directed to methods of screening for compounds

which modulate the expression of DNA or RNA encoding a NHL protein.

Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding NHL, or the function of the

5 NHL-based protein. Compounds that modulate the expression of DNA or RNA encoding NHL or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in
10 a standard sample. Kits containing NHL, antibodies to NHL, or modified NHL may be prepared by known methods for such uses.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of NHL. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend
15 themselves to the formulation of kits suitable for the detection and typing of NHL. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant NHL or anti-NHL antibodies suitable for detecting NHL. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates
20 or the like.

The assays described above can be carried out with cells that have been transiently or stably transfected with NHL. The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, protoplast fusion, and electroporation. Transfection is
25 meant to include any method known in the art for introducing NHL into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing NHL, and electroporation. The expression vector-containing cells are individually analyzed to determine whether they produce NHL protein. Identification of NHL expressing cells
30 may be done by several means, including but not limited to immunological reactivity with anti-NHL antibodies, labeled ligand binding, the presence of host cell-associated NHL activity.

The specificity of binding of compounds showing affinity for NHL is shown by measuring the affinity of the compounds for recombinant cells expressing NHL.

Expression of human NHL and screening for compounds that bind to NHL or that inhibit the binding of a known, radiolabeled ligand of NHL provides an effective method for the rapid selection of compounds with high affinity for NHL. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds
5 that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of NHL and may be peptides, proteins, or non-proteinaceous organic molecules.

Accordingly, the present invention is directed to methods for screening for
10 compounds which modulate the expression of DNA or RNA encoding a NHL protein as well as compounds which effect the function of the NHL protein. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of NHL. For example, Cascieri et al. (1992, *Molec. Pharmacol.* 41:1096-1099) describe a method for
15 identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells
20 in assay buffer containing a known radioactively labeled agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a
25 potential agonist or antagonist of the neurokinin receptor. Where binding of the substance such as an agonist or antagonist to is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

30 Therefore, the present invention includes assays by which modulators of NHL are identified. As noted above, methods for identifying agonists and antagonists are known in the art and can be adapted to identify compounds which effect *in vivo* levels of NHL. Accordingly, the present invention includes a method for determining whether a substance is a potential modulator of mammalian NHL levels that

comprises:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of NHL in the cells;
- (b) exposing the test cells to the substance;
- 5 (c) measuring the amount of binding of the substance to NHL;
- (d) comparing the amount of binding of the substance to NHL in the test cells with the amount of binding of the substance to control cells that have not been transfected with NHL or a portion thereof; wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance
- 10 is capable of binding to NHL.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

15 The assays described above can be carried out with cells that have been transiently or stably transfected with NHL. Transfection is meant to include any method known in the art for introducing NHL into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing NHL, and electroporation.

20 Where binding of the substance or agonist to NHL is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

Therefore, the specificity of binding of compounds having affinity for NHL shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to NHL or that inhibit the binding of a known, radiolabeled ligand of NHL to these cells provides an effective method for the rapid selection of compounds with high affinity for NHL. Such ligands need not

25 necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. It is also possible to construct assays wherein compounds are tested for an ability to modulate helicase activity in an *in vitro*- or *in vivo*- based assay.

30 Compounds identified by the above method again are likely to be agonists or

antagonists of NHL and may be peptides, proteins, or non-proteinaceous organic molecules. As noted elsewhere in this specification, compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding NHL, or by acting as an agonist or antagonist of the NHL receptor protein. Again, these compounds that
5 modulate the expression of DNA or RNA encoding NHL or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

10 Expression of NHL DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being
15 preferred.

Following expression of NHL in a host cell, NHL protein may be recovered to provide NHL protein in active form. Several NHL protein purification procedures are available and suitable for use. Recombinant NHL protein may be purified from cell lysates and extracts by various combinations of, or individual application of salt
20 fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography. In addition, recombinant NHL protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length NHL protein, or polypeptide fragments
25 of NHL protein.

Polyclonal or monoclonal antibodies may be raised against NHL or a synthetic peptide (usually from about 9 to about 25 amino acids in length) from a portion of NHL disclosed in SEQ ID NO:2. Monospecific antibodies to NHL are purified from mammalian antisera containing antibodies reactive against NHL or are prepared as
30 monoclonal antibodies reactive with NHL using the technique of Kohler and Milstein (1975, *Nature* 256: 495-497). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for NHL. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated

with NHL, as described above. Human NHL-specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with an appropriate concentration of NHL protein or a synthetic peptide generated from a portion of NHL with or without an immune adjuvant.

5 Preimmune serum is collected prior to the first immunization. Each animal receives between about 0.1 mg and about 1000 mg of NHL protein associated with an acceptable immune adjuvant. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The initial immunization consists of
10 NHL protein or peptide fragment thereof in, preferably, Freund's complete adjuvant at multiple sites either subcutaneously (SC), intraperitoneally (IP) or both. Each animal is bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. Those animals receiving booster injections are generally given an equal amount of
15 NHL in Freund's incomplete adjuvant by the same route. Booster injections are given at about three week intervals until maximal titers are obtained. At about 7 days after each booster immunization or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20°C.

Monoclonal antibodies (mAb) reactive with NHL are prepared by immunizing
20 inbred mice, preferably Balb/c, with NHL protein. The mice are immunized by the IP or SC route with about 1 mg to about 100 mg, preferably about 10 mg, of NHL protein in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0 and are rested for about 3 to about
25 30 weeks. Immunized mice are given one or more booster immunizations of about 1 to about 100 mg of NHL in a buffer solution such as phosphate buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic
30 lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody producing cells and myeloma cells are fused in polyethylene glycol, about 1000 mol. wt., at concentrations from about

30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells on about days 14, 18, and 21 and are screened for antibody
5 production by an immunoassay such as solid phase immunoradioassay (SPIRA) using NHL as the antigen. The culture fluids are also tested in the Ouchterlony precipitation assay to determine the isotype of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, 1973, Soft Agar Techniques, in *Tissue Culture Methods and Applications*, Kruse and
10 Paterson, Eds., Academic Press.

Monoclonal antibodies are produced *in vivo* by injection of pristine primed Balb/c mice, approximately 0.5 ml per mouse, with about 2×10^6 to about 6×10^6 hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified
15 by techniques known in the art.

In vitro production of anti- NHL mAb is carried out by growing the hybridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are purified by techniques known in the art.

Antibody titers of ascites or hybridoma culture fluids are determined by
20 various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of NHL in body fluids or tissue and cell extracts.

It is readily apparent to those skilled in the art that the above described
25 methods for producing monospecific antibodies may be utilized to produce antibodies specific for NHL peptide fragments, or a respective full-length NHL.

NHL antibody affinity columns are made, for example, by adding the antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the
30 agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3) and the cell

culture supernatants or cell extracts containing full-length NHL or NHL protein fragments are slowly passed through the column. The column is then washed with phosphate buffered saline until the optical density (A_{280}) falls to background, then the protein is eluted with 0.23 M glycine-HCl (pH 2.6). The purified NHL protein is then
5 dialyzed against phosphate buffered saline.

Pharmaceutically useful compositions comprising modulators of NHL may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a
10 pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA, modified NHL, or either NHL agonists or antagonists including tyrosine kinase activators or inhibitors.

Therapeutic or diagnostic compositions of the invention are administered to an
15 individual in amounts sufficient to treat or diagnose disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular.
20 The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are
25 described in a variety of texts, such as Remington's Pharmaceutical Sciences.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents may be desirable.

The present invention also has the objective of providing suitable topical, oral,
30 systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets,

capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or
5 intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds for the present invention can
10 be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

15 For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated;
20 the route of administration; the renal, hepatic and cardiovascular function of the patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision
25 in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

The present invention also relates to a non-human transgenic animal which is
30 useful for studying the ability of a variety of compounds to act as modulators of NHL, or any alternative functional NHL *in vivo* by providing cells for culture, *in vitro*. In reference to the transgenic animals of this invention, reference is made to transgenes and genes. As used herein, a transgene is a genetic construct including a gene. The transgene is integrated into one or more chromosomes in the cells in an animal by

methods known in the art. Once integrated, the transgene is carried in at least one place in the chromosomes of a transgenic animal. Of course, a gene is a nucleotide sequence that encodes a protein, such as one or a combination of the cDNA clones described herein. The gene and/or transgene may also include genetic regulatory elements and/or structural elements known in the art. A type of target cell for transgene introduction is the embryonic stem cell (ES). ES cells can be obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (Evans et al., 1981, *Nature* 292:154-156; Bradley et al., 1984, *Nature* 309:255-258; Gossler et al., 1986, *Proc. Natl. Acad. Sci. USA* 83:9065-9069; and Robertson et al., 1986 *Nature* 322:445-448). Transgenes can be efficiently introduced into the ES cells by a variety of standard techniques such as DNA transfection, microinjection, or by retrovirus-mediated transduction. The resultant transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The introduced ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal (Jaenisch, 1988, *Science* 240: 1468-1474). It will also be within the purview of the skilled artisan to produce transgenic or knock-out invertebrate animals (e.g., *C. elegans*) which express the NHL transgene in a wild type background as well in *C. elegans* mutants knocked out for one or both of the NHL subunits. These organisms will be helpful in further determining the dominant negative effect of NHL as well as selecting from compounds which modulate this effect.

The present invention also relates to a non-human transgenic animal which is heterozygous for a functional NHL gene native to that animal. As used herein, functional is used to describe a gene or protein that, when present in a cell or *in vitro* system, performs normally as if in a native or unaltered condition or environment. The animal of this aspect of the invention is useful for the study of the retinal specific expression or activity of NHL in an animal having only one functional copy of the gene. The animal is also useful for studying the ability of a variety of compounds to act as modulators of NHL activity or expression *in vivo* or, by providing cells for culture, *in vitro*. It is reiterated that as used herein, a modulator is a compound that causes a change in the expression or activity of NHL, or causes a change in the effect of the interaction of NHL with its ligand(s), or other protein(s). In an embodiment of this aspect, the animal is used in a method for the preparation of a further animal which lacks a functional native NHL gene. In another embodiment, the animal of this aspect is used in a method to prepare an animal which expresses a non-native NHL

gene in the absence of the expression of a native NHL gene. In particular embodiments the non-human animal is a mouse. In further embodiments the non-native NHL is a wild-type human NHL which is disclosed herein, or any other biologically equivalent form of human NHL gene as also disclosed herein.

5 In reference to the transgenic animals of this invention, reference is made to transgenes and genes. As used herein, a transgene is a genetic construct including a gene. The transgene is integrated into one or more chromosomes in the cells in an animal by methods known in the art. Once integrated, the transgene is carried in at least one place in the chromosomes of a transgenic animal. Of course, a gene is a
10 nucleotide sequence that encodes a protein, such as human or mouse NHL. The gene and/or transgene may also include genetic regulatory elements and/or structural elements known in the art.

Another aspect of the invention is a non-human animal embryo deficient for native NHL expression. This embryo is useful in studying the effects of the lack of
15 NHL on the developing animal. In particular embodiments the animal is a mouse. The animal embryo is also useful as a source of cells lacking a functional native NHL gene. The cells are useful in *in vitro* culture studies in the absence of NHL.

An aspect of this invention is a method to obtain an animal in which the cells lack a functional gene NHL native to the animal. The method includes providing a
20 gene for an altered form of the NHL gene native to the animal in the form of a transgene and targeting the transgene into a chromosome of the animal at the place of the native NHL gene. The transgene can be introduced into the embryonic stem cells by a variety of methods known in the art, including electroporation, microinjection, and lipofection. Cells carrying the transgene can then be injected into blastocysts
25 which are then implanted into pseudopregnant animals. In alternate embodiments, the transgene-targeted embryonic stem cells can be coincubated with fertilized eggs or morulae followed by implantation into females. After gestation, the animals obtained are chimeric founder transgenic animals. The founder animals can be used in further embodiments to cross with wild-type animals to produce F1 animals heterozygous for
30 the altered NHL gene. In further embodiments, these heterozygous animals can be interbred to obtain the non-viable transgenic embryos whose somatic and germ cells are homozygous for the altered NHL gene and thereby lack a functional NHL gene. In other embodiments, the heterozygous animals can be used to produce cells lines. In preferred embodiments, the animals are mice.

A further aspect of the present invention is a transgenic non-human animal which expresses a non-native NHL on a native NHL null background. In particular embodiments, the null background is generated by producing an animal with an altered native NHL gene that is non-functional, *i.e.* a knockout. The animal can be
5 heterozygous (*i.e.*, having a different allelic representation of a gene on each of a pair of chromosomes of a diploid genome) or homozygous (*i.e.*, having the same representation of a gene on each of a pair of chromosomes of a diploid genome) for the altered NHL gene and can be hemizygous (*i.e.*, having a gene represented on only one of a pair of chromosomes of a diploid genome) or homozygous for the non-native
10 NHL gene. In preferred embodiments, the animal is a mouse. In particular embodiments the non-native NHL gene can be a wild-type or mutant allele including those mutant alleles associated with a disease. In further embodiments, the non-native NHL is a human NHL. In a further embodiment the non-native NHL gene is operably linked to a promoter. As used herein, operably linked is used to denote a functional
15 connection between two elements whose orientation relevant to one another can vary. In this particular case, it is understood in the art that a promoter can be operably linked to the coding sequence of a gene to direct the expression of the coding sequence while placed at various distances from the coding sequence in a genetic construct.

20 An aspect of this invention is a method of producing transgenic animals having a transgene including a non-native NHL gene on a native NHL null background. The method includes providing transgenic animals of this invention whose cells are heterozygous for a native gene encoding a functional NHL protein and an altered native NHL gene. These animals are crossed with transgenic animals of
25 this invention that are hemizygous for a transgene including a non-native NHL gene to obtain animals that are both heterozygous for an altered native NHL gene and hemizygous for a non-native NHL gene. The latter animals are interbred to obtain animals that are homozygous or hemizygous for the non-native NHL and are homozygous for the altered native NHL gene. In particular embodiments, cell lines
30 are produced from any of the animals produced in the steps of the method.

The transgenic animals and cells of this invention are useful in the determination of the *in vivo* function of a non-native NHL in the central nervous system and in other tissues of an animal. The animals are also useful in studying the tissue and temporal specific expression patterns of a non-native NHL throughout the

animals. The animals are also useful in determining the ability for various forms of wild-type and mutant alleles of a non-native NHL to rescue the native NHL null deficiency. The animals are also useful for identifying and studying the ability of a variety of compounds to act as modulators of the expression or activity of a non-native NHL *in vivo*, or by providing cells for culture, for *in vitro* studies.

As used herein, a "targeted gene" or "Knockout" (KO) is a DNA sequence introduced into the germline of a non-human animal by way of human intervention, including but not limited to, the methods described herein. The targeted genes of the invention include nucleic acid sequences which are designed to specifically alter cognate endogenous alleles. An altered NHL gene should not fully encode the same NHL as native to the host animal, and its expression product can be altered to a minor or great degree, or absent altogether. In cases where it is useful to express a non-native NHL gene in a transgenic animal in the absence of a native NHL gene we prefer that the altered NHL gene induce a null lethal knockout phenotype in the animal. However a more modestly modified NHL gene can also be useful and is within the scope of the present invention.

A type of target cell for transgene introduction is the embryonic stem cell (ES). ES cells can be obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (Evans et al., 1981, *Nature* 292:154-156; Bradley et al., 1984, *Nature* 309:255-258; Gossler et al., 1986, *Proc. Natl. Acad. Sci. USA* 83:9065-9069; and Robertson et al., 1986 *Nature* 322:445-448). Transgenes can be efficiently introduced into the ES cells by a variety of standard techniques such as DNA transfection, microinjection, or by retrovirus-mediated transduction. The resultant transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The introduced ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal (Jaenisch, 1988, *Science* 240: 1468-1474).

The methods for evaluating the targeted recombination events as well as the resulting knockout mice are readily available and known in the art. Such methods include, but are not limited to DNA (Southern) hybridization to detect the targeted allele, polymerase chain reaction (PCR), polyacrylamide gel electrophoresis (PAGE) and Western blots to detect DNA, RNA and protein.

The following examples are provided to illustrate the present invention without, however, limiting the same hereto.

EXAMPLE 1

Characterization of DNA Molecules Encoding NHL

M68/DcR3 identification - The human osteoprotegerin (OPG) sequence (Acc. # U94332), which is a member of the TNFR-related family, was used to search Genbank using the programs TBLASTN and TFASTX3 to identify novel gene family members. Two EST sequences (GenBank Acc. # AA155701 and AA025672) were identified that showed sequence similarities to the cysteine repeats of the OPG sequence. These EST sequences were then used to identify additional EST sequences, which formed a single EST cluster (GenBank Acc. #s aa577603, aa603704, aa613366, aa158406, w67560, aa325843, aa155646, aa025673, aa514270, m91489). Two clones were further characterized, which were derived from colon tumor and germ cell tumor libraries (Research Genetics, Inc). DNA sequence analysis revealed two alternatively spliced forms of the 5'-end UTR of M68/DcR3. The M68/DcR3 open reading frame was confirmed by sequence analysis of clones obtained by PCR cloning from a normal human cDNA library (Clontech).

M68/DcR3 BAC identification and sequencing - To further delineate the gene structure of M68/DcR3, genomic DNA was obtained using a human "Down to the Well"™ genomic bacterial artificial chromosome (BAC) library (Genome Systems, Inc.) according to the manufacturer's protocol. Two sets of PCR primers, C68.36F: 5'-CACAGGTTTCAGCATGTTTGTGCGTC-3' (SEQ ID NO:4) and C68.275R: 5'-CACAGTCCCTGCTGGCCTCTGTCTA-3' (SEQ ID NO:5), and E68.715F: 5'-CAGGACATCTCCATCAAGAGGCTGC-3' (SEQ ID NO:6) and E68.972R: 5'-AATAAGAGGGGGCCAGGATCAGTGC-3' (SEQ ID NO:7), were used to carry out PCR reactions to identify positive wells that contained the full-length M68/DcR3 gene. The PCR conditions used were 94°C for 9min, 35 cycles of (94°C, 30 sec., 68°C 3 min.) followed by 72°C for 10 min. Two positive BAC clones were identified and characterized by restriction digestion and BAC-end sequence analyses, of which hbm168 was selected for shotgun sequencing.

A shot-gun library for BAC hbm168 was constructed using a conventional strategy. Briefly, two 150-ml bacterial cultures were combined and purified using a modified protocol of the plasmid-Maxi kit (QIAGEN) followed by CsCl gradient purification. After butanol extraction and isopropanol precipitation, BAC DNA was nebulized at 10 psi for 60 seconds to generate randomly sheared fragments.

Following ethanol precipitation, the fragments were end-repaired using T4 polymerase (Promega) and BstXI adaptors (Invitrogen) were ligated overnight. Removal of excess, unligated adaptors and size selection was performed using a cDNA sizing column (Life Technologies, Inc.) to generate genomic fragments in the size range of 5 1500 to 3000 bp. Adaptor ligated fragments were cloned into a modified pBlueScript SK⁺ vector (Stratagene) and transformed in XL2-Blue ultracompetent cells (Stratagene). Approximately 1000 clones were isolated, plasmids were purified using the Turbo miniprep kits (QIAGEN), and both plasmid ends were sequenced with the BigDye terminator kits (Perkin-Elmer). Sequence data were assembled using 10 Phred/Phrap/Consed where single-stranded and gap regions were closed using a directed sequencing strategy.

NHL identification and sequencing – The genomic clone for the NHL gene was obtained and sequenced. The transcript was identified through exon prediction using GRAIL2 and sequence alignment to a contiguous 4.5 kilobase region of 15 chromosome 4 (88% sequence identity). The complete exon structure of NHL was subsequently confirmed by RT-PCR analysis. The exon structure was confirmed by RT-PCR using polyA RNA from a human colorectal adenocarcinoma cell line, SW480 (Clontech). Primers were designed based on the genomic sequence that were predicted to be exons. RT-PCR reaction were carried out with SW480 polyA RNA 20 using standard conditions with TaqGold Enzyme at 94°C for 12min, 35 cycles of (94°C, 30 sec., 60°C, 30 sec., and 68°C 2-6 min.) followed by 68°C for 7 min. Most sequence confirmation was accomplished by RT-PCR, although first junction between exon 1 and 2 was confirmed by 5'RACE and junctions between exon 26-29 were by RCCA. The primers used were as follows:

25	<u>Junction of Exons</u>	<u>Confirmed by Primers</u>
	H01/H02	hdkw (5'RACE)
	H02/H03	hdiy,hdiz
	H03-H09	hdid,hdie,hdja,hdj b
	H09-H13	hdja,hdie
30	H13-H18	hdje,hdjf
	H18-H23	hdjg,hdjh
	H23-H26	hdji,hdj j
	H26-H29	hdkv,r543(RCCA)
	H29-H31	hdij,hdmu,hdnd,hdne

H31/H32	hdij,hdmu
H32/H34	hdip,hdil,hdmv,hdik,hdli
H34/H35	hdng,hdnh

- 5 HDID - 5'-GTGAATGGCATCCTGGAGAG-3' (SEQ ID NO:8);
 HDIE - 5'-GTCTCCAGGCAGCTCAACAG-3' (SEQ ID NO:9);
 HDIJ - 5'-ACCCTGTCCCTCCTGTCTGA-3' (SEQ ID NO:10);
 HDIY - 5'-AGACCCTAAGATGTTCCGAG-3' (SEQ ID NO:11);
 HDIZ - 5'-GATGACCTGTGTGAGTTGCG-3' (SEQ ID NO:12);
 10 HDJA - 5'-CGCAACTCACACAGGTCATC-3' (SEQ ID NO:13);
 HDJB - 5'-GGAGTCAGGTCAAAGGATGC-3' (SEQ ID NO:14);
 HDJC - 5'-GCATCCTTTGACCTGACTCC-3' (SEQ ID NO:15);
 HDJD - 5'-GGTCTGAAACGTGATCTGGG-3' (SEQ ID NO:16);
 HDJE - 5'-CCCAGATCACGTTTCAGACC-3' (SEQ ID NO:17);
 15 HDJF - 5'-CGATGATGTGTGGGTTCTCC-3' (SEQ ID NO:18);
 HDJG - 5'-GGAGAACCCACACATCATCG-3' (SEQ ID NO:19);
 HDJH - 5'-CGTGTCTGAGAAGTCCAGCC-3' (SEQ ID NO:20);
 HDJI - 5'-GGCTGGACTTCTCAGACACG-3' (SEQ ID NO:21);
 HDJJ - 5'-ACAGCATCTTCTCCACGCAC-3' (SEQ ID NO:22);
 20 HFMU - 5'-AGTCCTCTGGCTTTGCAGTG-3' (SEQ ID NO:23);
 HDKV - 5'-TGTGCGTGGAGAAGATGCTG-3' (SEQ ID NO:24);
 HDKW - 5'-GGCTGGAAAGGGAAGTCTAC-3' (SEQ ID NO:25);
 HDND - 5'-TGGTTCAGGTGCTCTTGGGG-3' (SEQ ID NO:26);
 HDNE - 5'-CGTGAAGCAGGAGTTGAGCC-3' (SEQ ID NO:27);
 25 HDIK - 5'-ATCTTGCTCTGGGTCTTCCC-3' (SEQ ID NO:28);
 HDIL - 5'-CACTGCAAAGCCAGAGGACT-3' (SEQ ID NO:29);
 HDIP - 5'-ATAAGCAAGACGACGACCTC-3' (SEQ ID NO:30);
 HDLI - 5'-CTATTCTGTTGGGTGGGTTC-3' (SEQ ID NO:31);
 HDMV - 5'-CGTGCCTCCTGTGCTTACCC-3' (SEQ ID NO:32);
 30 HDNG - 5'-CAGACCCCAAGGTAGCTCAG-3' (SEQ ID NO:33);
 HDNH - 5'-GGAAGACCCAGAGCAAGATC-3' (SEQ ID NO:34).

Amplified product were subject to direct sequencing after purification from an agarose gel or cloned into a TOPO PCR cloning vector (Invitrogen) for sequencing.

Multiple sequence alignment of NHL to known helicases showed that NHL contains
5 all the seven critical helicase domains. BLAST analysis of the predicted 1,219 amino acid sequence (see Figure 2, SEQ ID NO:2) reveal an approximately 26% sequence identity and 48% sequence similarity to the RAD3/ERCC2 gene family of DNA
10 helicases (see Figure 3). Review of this sequence data shows that two partial human cDNA clones (Acc. No. a1080127 and ab029011) are deposited. No. a1080127 covers exon 25-35 while ab029011 covers exons 9-35. Ab029011 starts at amino acid 240 of the full length human NHL protein disclosed herein, but also differs at exon 35 and appears to be a fusion transcript with M68. This cDNA was isolated from brain tissue, which has been known to express rare transcripts.

15

EXAMPLE 2

Northern Analysis of human NHL Expression

Messenger RNA (mRNA) obtained from human brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood leukocytes. Two μg of polyA⁺ RNA were run on each lane a denaturing
20 formaldehyde 1% agarose gel, and transferred to a charged-modified nylon membrane. The probe was made using a 733 bp fragment derived from 1174-1907 nt of the NHL cDNA. This fragment was labeled via the ³²P dCTP random priming method (Ambion). Hybridization was carried in ExpressHyb (Clontech) according to the manufacturer's protocol except for the final wash, which was at 55°C. Membranes
25 were exposed to X-ray film with intensifying screen at -80°C overnight. The Northern data is presented in Figure 4. Note hybridization of the NHL probe to an approximately 4.4 kb transcript. The 7.5 kb transcript may suggest an alternative splicing of the NHL RNA.

EXAMPLE 3

Chromosomal localization

To map the position of M68/NHL in the human genome, primers C68.36F and
5 C68.275R, were used to carry out PCR reactions to 93 clones of the MIT GeneBridge
4 panel (Research Genetics) and results were submitted to MIT for analysis.
M68/DcR3 was mapped to the extreme telomere of chromosome 20, at 20q13.3, 28cR
from D20S173 with a lod score of 13. An analogous procedure was also carried out
with the 83 clones of the Stanford G3 radiation hybrid panel, with PCR results
10 submitted to the Stanford Genome Center for analysis. Analysis using another pair of
PCR primers specific to NHL yielded the same result. For fluorescence in situ (FISH)
analysis, the normal human male fibroblast cell line, L136 (Coriell Cell Repository,
Camden, NJ) was arrested in mitosis with colcemid (10 μ g/ml). A human
chromosome 20 α -satellite probe (Vysis, Downers Grove, IL) was directly labeled
15 with Spectrum Orange dUTP and was used to identify chromosome 20. The M68
BAC clone was directly labeled with SpectrumGreen dUTP by nick translation
(Vysis). Slides were counterstained with DAPI stain and viewed under an Olympus
microscope with narrow blue and DAPI/TRITC filters. Fifty metaphase cells were
scored to verify that the M68 probe was located on the same chromosome as the
20 Human Chromosome 20 probe. Radiation hybrid chromosomal mapping reconfirms
that it is linked to M68 locus, at 20q13.3.

WHAT IS CLAIMED IS:

1. A purified DNA molecule encoding a mammalian NHL protein.

2. A purified DNA molecule of claim 1 encoding a human NHL protein

5 which comprises the amino acid sequence

MPKIVLNGVT VDFPFQPYKC QQEYMTKVLE CLQQKVNIGL ESPTGTGKTL CLLCTTLAWR
 EHLRDGISAR KIAERAQGEL FPDRLSSWG NAAAAAGDPI ACYTDIPKII YASRTHSQLT
 QVINELRNTS YRPKVCVLGS REQLCIHPEV KKQESNHLQI HLCRKKVASR SCHFYNNVEE
 KSLEQELASP ILDIEDLVKS GSKHRVCPYY LSRNLKQQAD IIFMPYNYLL DAKSRRAHNI
 10 DLKGTVVIFD EAHNVEKMCE ESASFDLTPH DLASGLDVID QVLEEQTCAA QQGEPHPEFS
 ADSPPSGLNM ELEDIAKLKM ILLRLEGAID AVELPGDDSG VTKPGSYIFE LFAEAQITFQ
 TKGCILDSDL QIIQHLAGRA GVFTNTAGLQ KLADIIQIVF SVDPSEGSFG SPAGLGALQS
 YKVHIHPDAG HRRTAQRSDA WSTTAARKRG KVLSYWCFSP GHSMHELVRQ GVRSLILTSG
 TLAPVSSFAL EMQIPFPVCL ENPHIIDKHQ IWVGVPVPRGP DGAQLSSAFD RRFSEECSS.
 15 LGKALGNIR VVPYGLLIFF PSYPVMEKSL EFWRARDLAR KMEALKPLFV EPRSKGSFSE
 TISAYYARVA APGSTGATFL AVCRGKASEG LDFSDTNGRG VIVTGLPYPP RMDPRVVLKM
 QFLDEMKGQG GAGGQFLSGQ EWYRQQASRA VNQAIGRVIR HRQDYGAVFL CDHRFAFADA
 RAQLPSWVRP HVRVYDNFGH VIRDAVQFFR VAERTMPAPA PRATAPSVRG EDAVSEAKSP
 GPFFSTRKAK SLDLHVPSLK QRSSGSPAAG DPESSLCVEY EQEPVPARQR PRGLLALEH
 20 SEQRAGSPGE EQAHSCSTLS LLSEKRPAEE PRGGRKKIRL VSHPEEPVAG AQTDRAKLFM
 VAVKQELSQA NFATFTQALQ DYKGSDDFAA LAACLGPLFA EDPKKHNLQ GFYQFVRPHH
 KQQFEEVCIQ LTGRGCGYRP EHSIPRRQRA QPVLDPGTGR APDPKLTVST AAAQQLDPQE
 HLNQGRPHLS PRPPPTGDPG SQPQWGSQVP RAGKQGHAV SAYLADARRA LGSAGCSQLL
 AALTAYKQDD DLDKVLAVLA ALTTAKPEDF PLLHRFSMFV RPHHKQRFSS TCTDLTGRPY
 25 PGMEPPGPQE ERLAVPPVLT HRAQQPGPSR SEKTGKTQSK ISSFLRQSPA GTVGAGGEDA
 GPSQSSGPPH GPAASEWGL* (SEQ ID NO:2).

3. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 2.

30

4. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 3.

5. A process for expressing a NHL protein in a recombinant host cell, comprising:

- (a) transfecting the expression vector of claim 3 into a suitable host cell; and,
 5 (b) culturing the host cells of step (a) under conditions which allow expression of said NHL protein from said expression vector.

6. A purified DNA molecule encoding a human NHL protein which consists of the amino acid sequence

```

10  MPKIVLNGVT VDFPFQPYKC QQEYMTKVLE CLQQKVNIGIL ESPTGTGKTL CLLCTTLAWR
    EHLRDGISAR KIAERAQGEL FPDRLSSWG NAAAAAGDPI ACYTDIPKII YASRTHSQLT
    QVINELRNST YRPKVCVLGS REQLCIHPEV KKQESNHLQI HLCRKKVASR SCHFYNNVEE
    KSLEQELASP ILDIEDLVKS GSKHRVCPY LSRNLKQQAD IIFMPYNYLL DAKSRRAHNI
    DLKGTVVIFD EAHNVEKMCE ESASFDLTPH DLASGLDVID QVLEEQTCAA QQGEPHPEFS
15  ADSPSPGLNM ELEDIAKLKM ILLRLEGAID AVELPGDDSG VTKPGSYIFE LFAEAQITFQ
    TKGCILDSLD QIIQHLAGRA GVFTNTAGLQ KLADIIQIVF SVDPSEGSFG SPAGLGALQS
    YKVHIHPDAG HRRTAQRSDA WSTTAARKRG KVLSYWCFSP GHSMHELVRQ GVRSLILTSG
    TLAPVSSFAL EMQIPFPVCL ENPHIIDKHQ IWGVVPRGP DGAQLSSAFD RRFSEECCLSS
    LGKALGNIR VVPYGLLIFF PSYPVMEKSL EFWRARDLAR KMEALKPLFV EPRSKGSFSE
20  TISAYYARVA APGSTGATFL AVCRGKASEG LDFSDTNGRG VIVTGLPYPP RMDPRVVLKM
    QFLDEMKGQG GAGGQFLSGQ EWYRQQASRA VNQAIGRVIR HRQDYGAVFL CDHRFAFADA
    RAQLPSWVRP HVRVYDNFGH VIRDVAQFFR VAERTMPAPA PRATAPSVRG EDAVSEAKSP
    GPPFFSTRKAK SLDLHVPSLK QRSSGSPAAG DPESLSCVEY EQEPVPARQR PRGLLAALAH
    SEQRAGSPGE EQAHSCSTLS LLSEKRPAEE PRGGRKKIRL VSHPEEPVAG AQTDRAKLFM
25  VAVKQELSQA NFATFTQALQ DYKGSDDFAA LAACLGPLFA EDPKKHNLQ GFYQFVRPHH
    KQQFEVCIQ LTGRGCGYRP EHSIPRRQRA QPVLDPGTGR APDPKLTVST AAAQQQLDPQE
    HLNQGRPHLS PRPPPTGDPG SQPQWGSQVP RAGKQGHAV SAYLADARRA LGSAGCSQLL
    AALTAYKQDD DLDKVLAVLA ALTAKPEDF PLLHRFSMFV RPHHKQRFSQ TCTDLTGRPY
    PGMEPPGPQE ERLAVPPVLT HRAPQGPSR SEKTGKTQSK ISSFLRQRPA GTVGAGGEDA
30  GPSQSSGPPH GPAASEWGL* (SEQ ID NO:2) .
  
```

7. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 6.

8. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 7.
9. A process for expressing a NHL protein in a recombinant host cell,
5 comprising:
 (a) transfecting the expression vector of claim 7 into a suitable host cell; and,
 (b) culturing the host cells of step (a) under conditions which allow expression of said NHL protein from said expression vector.
10. A purified DNA molecule which comprises the nucleotide sequence as set forth in SEQ ID NO:1.
11. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 10.
15
12. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 11.
13. A purified DNA molecule which consists of the nucleotide sequence as set forth in SEQ ID NO:1.
20
14. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 13.
15. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 14.
25
16. A purified DNA molecule of claim 13 which consists of the nucleotide sequence from about nucleotide 828 to about nucleotide 4587, as set forth in SEQ ID NO:1.
30
17. An expression vector for expressing a NHL protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 16.

18. A host cell which expresses a recombinant NHL protein wherein said host cell contains the expression vector of claim 17.

19. A substantially purified NHL protein which comprises the amino acid
5 sequence as set forth in SEQ ID NO:2.

20. A substantially purified NHL protein which consists of the amino acid sequence as set forth in SEQ ID NO:2.

10 21. A substantially purified NHL protein which comprises the amino acid sequence as set forth in SEQ ID NO:2, wherein said protein is a product of a DNA expression vector comprising SEQ ID NO:1 and contained within a recombinant host cell.

15 22. A method of identifying modulators of NHL activity, comprising:
(a) combining a test compound with a NHL protein, wherein NHL comprises the amino acid sequence as set forth in SEQ ID NO:2; and,
(b) measuring the effect of the test compound on the NHL protein.

20 23. An isolated DNA molecule which comprises the nucleotide sequence as set forth in SEQ ID NO:3.

24. An isolated DNA molecule of claim 20 which comprises from about nucleotide 47000 to about nucleotide 85500 of SEQ ID NO:3.

25 25. An isolated DNA molecule of claim 23 which comprises from about nucleotide 47095 to about nucleotide 85316 of SEQ ID NO:3.

26. A substantially purified NHL protein of claim 21 wherein said protein
30 is a product of a DNA expression vector comprising from about nucleotide 828 to nucleotide 4587, as set forth in SEQ ID NO:1, and contained within a recombinant host cell.

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AGTCAGCCCT GCTGCCAGCC AGTGCCGGGT GCTGGGGACT CAGGGAGGCC CGCCGGGACC ACTGCCGGAC
AGTGAGCCGA GCAGAAGCTG GAACGCAGGA GAGGAAGGAG AGGGGGCGGT CAGGGCTCTC AGGAGCCGGG
TCCTGGGCAA GCGCGAGCCG TTTTCAAATT TTCAGGAAAG CGGTCGGCTC AACTCGAGC AGTAAAAAGA
TGCTCTGGG GAGGAGGCC GTGCAGCTCT CCGGGCAATG GTGGTGGCTC GGCCTAGAGA GCGGTAGTG
GAACGCAGAC CCTGGTGGGG GAATGACATC AAGGGAGGAG ACGGGCGGGA CCCCAGATTT CTGCCTGTGG
GCGATGGAAG TGAGGTTTAC TGGCCAGCGG AGCCGGACAC AGAACGCGCA AAACGCCGTG TAGGCCTGGA
GGAGCCGAAG AGCAGGCGGA CCCCTCCGC GGGGGAACAG TTTCCGCCGG GAGCACAAG CAACGGACCG
GAAGTGGGGG GCGGAAGTGC AGTGGGCTCA GCGCCGACTG CGCGCCTCTG CCCGCGAAAA CTCTGAGCTG
GCTGACAGCT GGGGACGGGT GCGGGCCCTC GACTGGAGTC GGTGAGTTC CTGAGGGACC CCGGTTCTGG
AAGGTTCCGC GCGGAGACAA GTGAGCAGTC TGTGCCATAG GGATTCTCGA AGAGAACAGC GTTGTGTCCC
AGTGACATG CTCGCATCGC TTACCAGGAG TGCCCGAGAC CCTAAGATGT TCGGAGTGGT TTTTTCGCAC
AGACCGAAT AGCCTGCCCC TCAGCCACGC TCTGTGCCCT TCTGAGAACA GGCTGATATG CCAAGATAG
TCCTGAATGG TGTGACCGTA GACTTCCCTT TCCAGCCCTA CAAATGCCAA CAGGAGTACA TGACCAAGGT
CCTGGAATGT CTGCAGCAGA AGGTGAATGG CATCCTGGAG AGCCCTACGG GTACAGGGAA GACGCTGTGC
CTGCTGTGCA CCACGCTGGC CTGGCGAGAA CACCTCCGAG ACGGCATCTC TGCCCGCAAG ATTGCCGAGA
GGGCGCAAGG AGAGCTTTTC CCGGATCGGG CTTGTGCATC CTGGGGCAAC GCTGCTGTG CTGCTGGAGA
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GTCATCAACG AGCTTCGGAA CACCTCCTAC CGGCCTAAGG TGTGTGTGCT GGGTCCCCGG GAGCAGCTGT
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AAGTCGCTCC TGTCAATTTCT ACAACAACGT AGAAGAAAA AGCCTGGAGC AGGAGCTGGC CAGCCCCATC
CTGGACATTG AGGACTTGGT CAAGAGCGGA AGCAAGCACA GGGTGTGCC TTTACTACCTG TCCCGGAACC
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CAACATTGAC CTGAAGGGGA CAGTCGTGAT CTTTGACGAA GCTCACAACG TGGAGAAGAT GTGTGAAGAA
TCGGCATCCT TTGACCTGAC TCCCCATGAC CTGGCTTCAG GACTGGACGT CATAGACCAG GTGCTGGAGG
AGCAGACCAA GGCAGCGCAG CAGGGTGAGC CCCACCCGGA GTTCAGCGCG GACTCCCCCA GCCCAGGGCT
GAACATGGAG CTGGAAGACA TTGCAAAGCT GAAGATGATC CTGCTGCGCC TGGAGGGGG CATCGATGCT
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ACGTGCTGGA GTGTTACCA ACACGGCCGG ACTGCAGAAG CTGGCGGACA TTATCCAGAT TGTGTTCACT
GTGGACCCCT CCGAGGGCAG CCCTGGTTCC CCAGCAGGGC TGGGGGCTT ACAGTCCTAT AAGGTGCACA
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GCGAGGGAAG GTGCTGAGCT ACTGGTGCTT CAGTCCCGGC CACAGCATGC ACGAGCTGGT CCGCCAGGGC
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CTTTCCAGT CTGCCTGGAG AACCCACACA TCATCGACAA GCACCAGATC TGGGTGGGG TCGTCCCCAG
AGGCCCCGAT GGAGCCAGT TGAGCTCCGC GTTTGACAGA CGGTTTTCCG AGGAGTGCTT ATCCTCCCTG
GGGAAGGCTC TGGGCAACAT CGCCCGCGTG GTGCCCTATG GGCTCCTGAT CTTCTTCCCT TCCTATCCTG
TCATGGAGAA GAGCCTGGAG TTCTGGCGGG CCCGCGACTT GGCCAGGAAG ATGGAGGCGC TGAAGCCGCT
GTTTGTGGAG CCCAGGAGCA AAGGCAGCTT CTCCGAGACC ATCAGTGCTT ACTATGCAAG GGTGCGCGC
CCTGGGTCCA CCGGCGCCAC CTTCTGGCG GTCTGCCGGG GCAAGGCCAG CGAGGGGCTG GACTTCTCAG
ACACGAATGG CCGTGGTGTG ATTGTCACGG GCCTCCCGTA CCCCCACGC ATGGACCCC GGGTTGTCTT
CAAGATGCAG TTCCTGGATG AGATGAAGGG CCAGGTGGG GCTGGGGGCC AGTTCTCTC TGGCAGGAG
TGGTACCGGC AGCAGGCGTC CAGGGCTGTG AACCAGGCCA TCGGGCGAGT GATCCGGCAC CGCCAGGACT
ACGGAGCTGT CTTCTCTGT GACCACAGGT TCGCCTTTGC CGACGCAAGA GCCCACTGC CCTCTGGGT
GCGTCCCCAC GTCAGGGTGT ATGACAACTT TGGCCATGTC ATCCGAGACG TGGCCAGTT CTTCCGTGT
GCCGAGCGAA CTATGCCAGC GCCGGCCCC CGGGCTACAG CACCCAGTGT GCGTGGAGAA GATGCTGTCA
GCGAGGCCAA GTCGCTGGC CCCTTCTTCT CCACCAGGAA AGCTAAGAGT CTGGACCTGC ATGTCCCCAG
CCTGAAGCAG AGGTCTCAG GGTACACAGC TGCCGGGGAC CCCGAGAGTA GCCTGTGTGT GGAGTATGAG
CAGGAGCCAG TTCCTGCCCG GCAGAGGCC AGGGGGTGC TGGCCGCCCT GGAGCACAGC GAACAGCGGG

FIG. 1A

SUBSTITUTE SHEET (RULE 26)

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CGGGGAGCCC TGGCGAGGAG CAGGCCACACA GCTGCTCCAC CCTGTCCCTC CTGTCTGAGA
AGAGGCCGGC AGAAGAACCG CGAGGAGGGA GGAAGAAGAT CCGGCTGGTC AGCCACCCGG
AGGAGCCCGT GGCTGGTGCA CAGACGGACA GGGCCAAGCT CTTTCATGGT GCCGTGAAGC
AGGAGTTGAG CCAAGCCAAC TTTGCCACCT TCACCCAGGC CCTGCAGGAC TACAAGGGTT
CCGATGACTT CGCCGCCCTG GCCGCCTGTC TCGGCCCCCT CTTTGCTGAG GACCCCAAGA
AGCACAACT GCTCCAAGGC TTCTACCAGT TTGTGCGGCC CCACCATAAG CAGCAGTTTG
AGGAGGTCTG TATCCAGCTG ACAGGACGAG GCTGTGGCTA TCGGCCTGAG CACAGCATTC
CCCGAAGGCA GCGGGCACAG CCGGTCCTGG ACCCACTGG AAGAACGGCG CCGGATCCCA
AGCTGACCGT GTCCACGGCT GCAGCCACAG AGCTGGACCC CCAAGAGCAC CTGAACCAGG
GCAGGCCCA CCTGTGCCCC AGGCCACCCC CAACAGGAGA CCCTGGCAGC CAACCACAGT
GGGGGTCTGG AGTGCCCAAG GCAGGGAAGC AGGGCCAGCA CGCCGTGAGC GCCTACCTGG
CTGATGCCCG CAGGGCCCTG GGGTCCGCGG GCTGTAGCCA ACTCTTGCCA GCGCTGACAG
CCTATAAGCA AGACGACGAC CTCGACAAGG TGCTGGCTGT GTTGGCCGCC CTGACCACTG
CAAAGCCAGA GGAATTCCCC CTGCTGCACA GGTTGAGCAT GTTTGTGCGT CCACACCACA
AGCAGCGCTT CTCACAGACG TGACAGACC TGACCGGCCG GCCCTACCCG GGCATGGAGC
CACCGGGACC CCAGGAGGAG AGGCTTGCCG TGCTCCTGT GCTTACCCAC AGGGCTCCCC
AACCAGGCC CTACCGGTCC GAGAAGACCG GGAAGACCCA GAGCAAGATC TCGTCTTCC
TTAGACAGAG GCCAGCAGGG ACTGTGGGGG CGGGCGGTGA GGATGCAGGT CCCAGCCAGT
CCTCAGGACC TCCCCACGGG CCTGCAGCAT CTGAGTGGGG CCTCTAGGAT GTGCCAGCC
TGCCACACCG CCTCCAGGAA GCAGAGCGTC ATGCAGGTCT TCTGGCCAGA GCCCCAGTGA
GTGCCACGG AGGCCCCCAG CACACCAAC GTGGCTTGAT CACCTGCCTG TCCAGCTCTG
GTGGGCCAAG AACCACCCA ACAGAATAGG CCAGCCCATG CCAGCCGGCT TGGCCCGCTG
CAGGCCTCAG GCAGGCGGGG CCCATGGTTG GTCCCTGCGG TGGGACCGGA TCTGGGCCTG
CCTCTGAGAA GCCCTGAGCT ACCTTGGGGT CTGGGGTGGG TTTCTGGGAA AGTGCTTCCC
CAGAACTTCC CTGGCTCCTG GCCTGTGAGT GGTGCCACAG GGGCACCCCA GCTGAGCCCC
TCACCGGGAA GGAGGAGACC CCCGTGGGCA CGTGTCCACT TTTAATCAGG GGACAGGGCT
CTCTAATAAA GCTGCTGGCA GTGCCC (SEQ ID NO:1).

FIG.1B

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MPKIVLNGVT VDFPFQPYKC QQEYMTKVLE CLQQKVNGIL ESPTGTGKTL CLLCTTLAWR
EHLRDGISAR KIAERAQGEL FPDRLSSWG NAAAAAGDPI ACYTDIPKII YASRTHSQLT
QVINELRNTS YRPKVCVLGS REQLCIHPEV KKQESNHLQI HLCRKKVASR SCHFYNNVEE
KSLEQELASP ILDIEDLVKS GSKHRVCPYY LSRNLKQQAD IIFMPYNYLL DAKSRRAHNI
DLKGTVVIFD EAHNVEKMCE ESASFDLTPH DLASGLDVID QVLEEQTAA QQGEHPPEFS
ADSPSPGLNM ELEDIAKLKM ILLRLEGAI D AVELPGDDSG VTKPGSYIFE LFAEAQITFQ
TKGCILDSLD QIIQHLAGRA GVFTNTAGLQ KLADIIQIVF SVDPSEGSPG SPAGLGALQS
YKVHIHPDAG HRRTAQRSDA WSTTAARKRG KVLSYWCFSF GHSMHELVRO GVRSLILTSG
TLAPVSSFAL EMQIPFPVCL ENPHIIDKHQ IWVGVPVPRG DGAQLSSAFD RRFSEECSS
LGKALGNIAR VVPYGLLIFF PSYPVMEKSL EFWRRDLAR KMEALKPLFV EPRSKGSFSE
TISAYYARVA APGSTGATFL AVCRGKASEG LDFSDTNGRG VIVTGLPYPP RMDPRVVLKM
QFLDEMGQG GAGGQFLSGQ EWYRQQASRA VNQAIGRVIR HRQDYGAVFL CDHRFAFADA
RAQLPSWVRP HVRVYDNFGH VIRDVAQFFR VAERTMPAPA PRATAPSVRG EDAVSEAKSP
GPFFSTRKAK SLDLHVPSLK QRSSGSPAAG DPESLCEVEY EQEPVPARQR PRGLLALEH
SEQRAGSPGE EQAHSCSTLS LLSEKRPAEE PRGGRKKIRL VSHPEEPVAG AQTDRAKLFM
VAVKQELSQA NFATFTQALQ DYKGSDDFAA LAACLGPLFA EDPKKHNLQ GFYQFVRPHH
KQQFEEVCIQ LTGRGCGYRP EHSIPRRQRA QPVLDPGTGR APDPKLTVST AAAQQLDPQE
HLNQGRPHLS PRPPPTGDPG SQPQWGSVP RAGKQGHAV SAYLADARRA LGSAGCSQLL
AALTAYQDD DLDKVLAVLA ALTAKPEDF PLLHRFSMFV RPHHKQRFSS TCTDLTGRPY
PGMEPPGPQE ERLAVPPVLT HRAPOGPSR SEKTGKTQSK ISSFLRQSPA GTVGAGGEDA
GPSQSSGPPH GPAASEWGL* (SEQ ID NO:2).

FIG.2

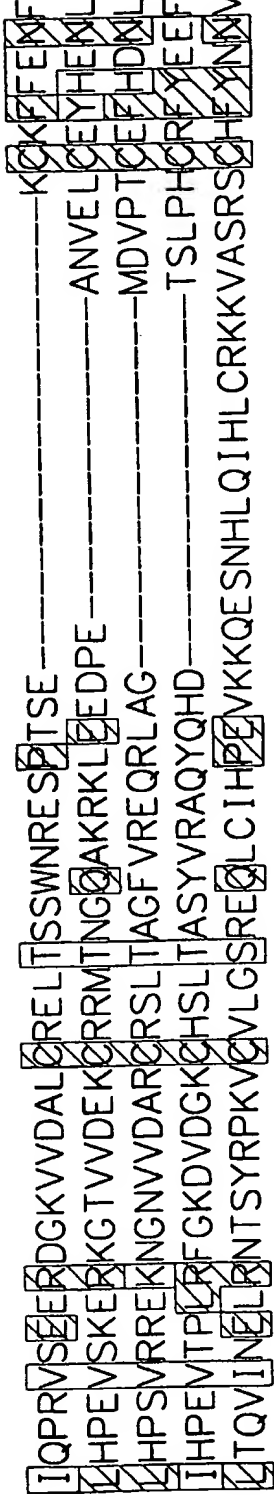
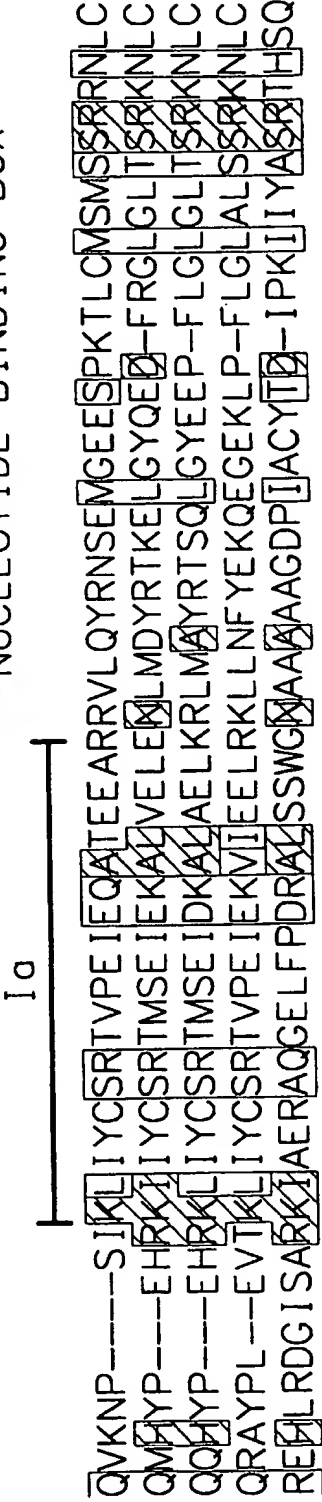
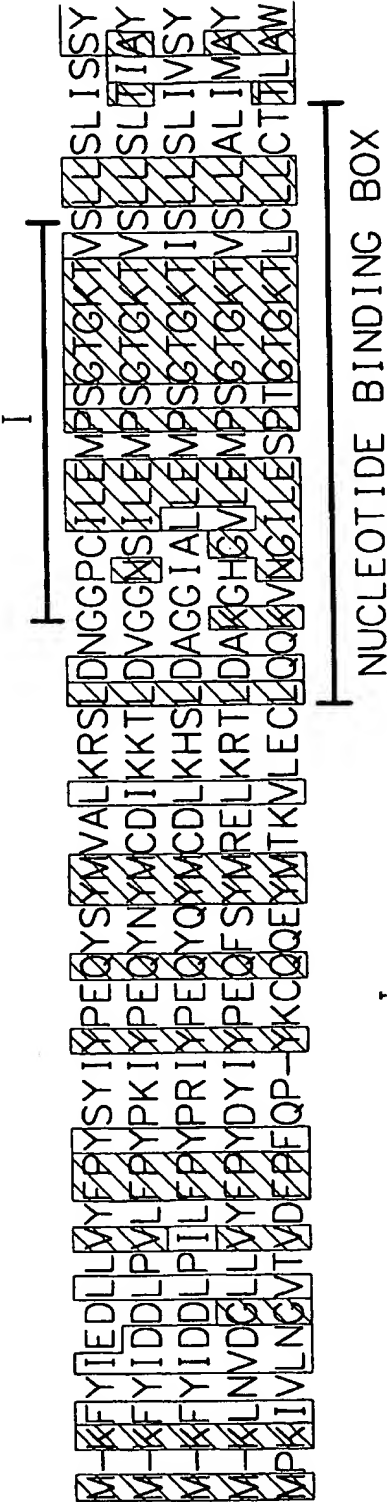


FIG. 3A

RepD
RAD3
RAD15
XP_GroupD
NHL

S	N	G	K	E	I	L	-	E	G	V	S	L	E	D	L	K	E	Y	L	K	H	Q	M	C	P	Y	F	L	S	R	H	M	L	N	F	A	N	I	V	I	F	S	Q	Y	L	L	D	P	K	I	A	S	L	I		
Y	N	I	E	V	E	D	Y	P	K	G	V	F	S	F	E	K	L	K	Y	C	E	E	K	T	L	C	P	Y	F	I	V	R	M	I	S	L	O	N	I	I	Y	S	H	Y	L	L	D	P	K	I	A	E	R	V		
D	L	E	P	H	S	L	I	S	N	G	V	W	T	L	D	I	I	T	E	Y	G	E	K	T	T	R	C	P	Y	F	T	V	R	M	L	P	F	C	N	V	I	I	Y	S	H	Y	L	L	D	P	K	I	A	E	R	V
D	A	H	G	R	E	V	P	L	P	A	G	T	Y	N	L	D	L	K	A	L	G	R	R	Q	G	W	C	P	Y	F	L	A	R	Y	S	I	L	H	A	N	V	V	Y	S	H	Y	L	L	D	P	K	I	A	D	L	V
E	K	S	L	E	Q	E	K	A	S	P	I	L	D	I	E	D	L	V	K	S	G	S	K	H	R	V	C	P	Y	F	L	S	R	N	L	K	Q	Q	A	D	I	F	M	P	N	Y	L	L	D	A	K	S	R	R	A	H

RepD
RAD3
RAD15
XP_GroupD
NHL

II

S	S	S	F	P	S	N	S	I	V	F	D	E	A	H	N	I	D	N	V	C	I	N	A	L	S	I	N	I	D	N	K	L	D	T	S	S	K	N	I	A	K	I	N	K	Q	I	E	D	I	K	V	D	E	K	R	L	
S	N	E	V	S	K	D	S	I	V	F	D	E	A	H	N	I	D	N	V	C	I	E	S	L	S	L	D	L	T	D	A	L	R	R	A	T	R	G	A	N	A	L	D	E	R	I	S	E	V	R	K	V	D	S	Q	K	L
S	R	E	L	S	K	D	C	I	V	F	D	E	A	H	N	I	D	N	V	C	I	E	S	L	S	I	D	L	T	E	S	S	L	R	K	A	S	K	S	L	S	L	E	Q	K	V	N	E	V	K	S	D	S	K	K	L	
S	K	E	L	A	R	K	A	V	V	F	D	E	A	H	N	I	D	N	V	C	I	D	S	M	S	N	L	T	R	T	L	D	R	C	Q	G	N	L	E	T	L	Q	K	T	V	L	R	I	K	E	T	D	E	Q	R	L	
N	I	D	L	K	G	-	T	V	V	F	D	E	A	H	N	I	D	N	V	C	I	E	S	L	S	F	D	L	T	P	H	D	L	A	S	G	L	D	V	L	D	Q	V	L	E	E	Q	I	K	A	A	G	Q	Q	G	P	-

RepD
RAD3
RAD15
XP_GroupD
NHL

K	D	E	Y	Q	R	L	V	N	G	L	A	R	S	G	S	T	R	A	--	D	E	T	T	S	D	P	V	L	P	N	D	V	I	Q	E	A	V	P	G	N	I	R	K	P	S	I	F	I	S	L	L	R	R	V	D	Y	L			
Q	D	E	Y	E	K	L	V	Q	G	L	H	S	A	D	I	L	T	Q	E	E	P	F	V	E	T	P	V	L	P	Q	D	L	L	T	E	A	I	P	G	N	I	R	R	A	E	H	F	V	S	F	L	K	R	L	I	E	Y	L		
Q	D	E	Y	Q	K	L	V	R	G	L	Q	D	A	N	A	N	D	-	E	D	Q	F	M	A	N	P	V	L	P	E	D	V	L	K	E	A	V	P	G	N	I	R	R	A	E	H	F	I	A	F	L	K	R	F	V	E	Y	L		
R	D	E	Y	R	R	L	V	E	G	L	R	E	A	S	A	A	R	E	-	T	D	A	H	L	A	N	P	V	L	P	D	E	N	V	L	Q	E	A	V	P	G	S	I	R	T	A	E	H	F	L	G	F	L	R	R	L	L	E	Y	V
H	P	E	F	S	A	D	S	P	S	P	G	L	N	M	E	L	E	D	I	A	K	L	K	M	I	L	L	R	L	E	G	A	I	D	A	V	E	L	P	G	D	S	G	V	T	K	P	G	S	Y	I	F	E	L	F	A	E	A		

FIG. 3B

KSR LK SQML SE8 PLAF LGGY -- HATQ ISSRT RF CSSR LSSLLRTL RINDVNQFSG-I
KTR MKVLHV SETPKSFLQHK -- QLTIERKPRFC SERLSL LVRTLEVTEVEDFTA-L
KTR MKVLHV AETPTSFLQHK -- DITIDKKPRFCAERL TSLVRALQISLVEDFHS-L
KWR LRVQHVMQE8PPAFLSGLA -- QRMCIQRKPRFCAERL RSLHTLEITDLADFSP-L
QITFQTKGC L D8LDQI I QHLAGRAGV TINTAGLQK LADIIQIVF SVDPSGSPGSPAGL

REPD
RAD3
RAD15
XP_GroupD
NHL

SLIADFA TLVGTYN -- NGFLIIIEPY YQRQNNTYDQIFQFC L DASIGMKP IFDK-YR5V
KDIATFATL I STYE -- EGFLLIEPYE IENAAVPNPIMRFTCL DASIAIKPVFER-F5SV
QQVAFATLVATYE -- KGFILILEPFENATVPNPILRFSC L DASIAIKPVFER-F5SV
TLLANFATLVSTYA -- KGFTHIEPFDRTPTIANPILHFSMDASLAIKPVFER-F5SV
GALQ8YK VHIHPDAGHRTAQRSDAMS TARKRGKVL SYWCFSPGHSMEHLVRQGV8SL

REPD
RAD3
RAD15
XP_GroupD
NHL

III
VITSGT L SPLDIYTKMLNFRPTXVERLTMS LNRNCJCPCLITRGSDQISISITKFDVRSDT
IITSGT L SPLDMYPRMLNF KTVLQKSYAMT LAKSFLPMIITKGSQQVAISSRPEIRNDP
IITSGT L SPLDMYPKMLQFNTVMQESYGMS LARNCF LPMVITRGSDQVAISSKFEARNDP
IITSGT L SPLDIYPKI LDFHPVTMATFTMTLARVCLCPMIICRGNDQVAISSKFEITREDI
IITSGT L SPLDIYSSFALEMQIPFPVCLENPHIIDKHQJWVGVPYPCPDCAQLSSAFDRPFSE

REPD
RAD3
RAD15
XP_GroupD
NHL

FIG. 3C

REPD
RAD3
RAD15
XP_GroupD
NHL

AV	VR	NY	CA	LV	SA	VP	DP	GI	IC	FF	TS	SY	ME	QI	VS	VM	EM	GL	LN	NI	LT	NK	IL	IE	VE	TS	DP		
SI	VR	NY	CS	ML	VE	AK	IT	DP	DC	MV	FF	PS	LY	ME	SI	SM	QT	MG	IL	DE	VW	KH	KL	IL	VE	TP	DA		
SV	VR	NY	GN	IL	VE	FS	KI	IT	DP	DC	IV	AF	FP	SL	LY	ES	IV	SS	WQ	SM	IL	DE	VW	KY	KL	IL	VE	TP	DP
AV	IR	NY	GN	IL	EM	SA	VP	DP	GI	IC	FF	TS	QY	ME	ST	VS	WY	EQ	IL	EN	IQ	RN	KL	IL	VE	TP	QD	GC	
EC	LS	SL	CA	KL	GN	IR	VP	DP	GI	IC	FF	PS	LY	ME	KS	LE	FW	RD	IL	AR	KE	AL	KP	IL	VE	TP	RS	SK	

REPD
RAD3
RAD15
XP_GroupD
NHL

AES	AL	AL	QN	Y	KK	AC	DS	GR	CA	VL	LS	MA	R	G	K	V	SE	GI	DF	DN	QY	GR	CV	IL	YG	IP	Y	IN	TE	SK	V
QET	SL	AL	ET	Y	KK	AC	SN	GR	CA	IL	LS	MA	R	G	K	V	SE	GI	DF	DH	QY	GR	TV	ML	IG	IP	Y	QY	TE	S	R
HET	TL	AL	ET	Y	RA	AC	SN	GR	CA	VL	LS	MA	R	G	K	V	SE	GI	DF	DH	Y	GR	AV	IM	FG	IP	Y	QY	TE	S	R
AET	SV	AL	EK	Y	QE	AC	EN	GR	CA	IL	LS	MA	R	G	K	V	SE	GI	DF	VH	Y	GR	AV	IM	FG	IP	Y	VY	TE	S	R
GSF	ET	IS	AY	AR	VA	AP	GS	TC	AT	FL	AV	CR	KA	SE	GL	DF	SD	TN	GR	CV	IL	YG	IP	Y	QY	TE	S	R	PP	RM	DP

REPD
RAD3
RAD15
XP_GroupD
NHL

L	R	A	L	E	F	L	R	D	R	Y	Q	I	R	E	N	---	E	F	L	T	---	F	D	A	M	R	T	A	S	Q	C	V	G	R	V	T	R	G	K	S	D	Y	G	---	I	M	I	F	A	D	K	R	Y				
L	K	A	R	L	E	F	M	R	E	N	Y	R	I	R	E	N	---	D	E	L	S	---	F	D	A	M	R	H	A	A	Q	C	L	G	R	V	L	R	C	K	D	D	Y	G	---	M	M	L	A	D	R	R	E				
L	K	A	R	L	E	F	L	R	D	T	Y	Q	I	R	E	N	---	D	E	L	T	---	F	D	A	M	R	H	A	A	Q	C	L	G	R	V	L	R	C	K	D	D	H	G	---	I	M	L	A	D	K	R	Y				
L	K	A	R	L	E	Y	L	R	D	Q	F	Q	I	R	E	N	---	D	E	L	T	---	F	D	A	M	R	H	A	A	Q	C	V	G	R	A	T	R	C	K	T	D	Y	G	---	L	M	F	A	D	K	R	E				
V	V	L	K	M	Q	F	L	D	E	M	K	G	Q	G	A	G	G	Q	F	L	S	---	G	Q	E	W	R	Q	A	S	R	A	V	N	Q	A	I	G	R	V	T	R	H	R	Q	D	Y	G	---	A	V	F	L	C	D	R	E

FIG. 3D

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NRLKRNKLPQWILQFC-QPQHNLSTDMAISLKITFLREMGQPFSEEQLGKSLWSLEH
SR--KRSQPKWIAQGL-SDADNLSTDMAISNTKQFLRTMAQPTDPKDQEGMSVWSYED
GRSDKRTKLPKWIIQQYI-TEGATNLSTDMSLALAKKFLRTMAQPFRTASDQEGISWWSLDD
ARGDKRGKLPKWIIQEL-TDANLNLTVDEGVQVAKYFLRQMAQPFHREDQLGLSLLSLEQ
AFADARAQLPSMVRPCHVRVYDNFCHVIRDVAGQFRVAERTMPADAPBATAAPSVRGEDAVS

REPD
RAD3
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NHL

VEKQSTSKPPQQNSAINSTITTTTITTTTITSETHLT (SEQ ID NO:35)
LIKHNQNS--RKDQCGFIENENKEGEQDEDEDEIEMQ (SEQ ID NO:36)
LLIHQK---KALSAALQSKHEMDIDVVET (SEQ ID NO:37)
LESEETL--KRIEQIAQQL (SEQ ID NO:38)
EAKSPGPFSTRKAKSLDLHVPSLKQRSSGSPAAGDPRESSLCVEYEQEPVPARQRPRL

REPD
RAD3
RAD15
XP_GroupD
NHL

FIG. 3E

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AALEHSEQRAGSPGEEQAHSCSTLSLLSEKRPAEEPRGGRKKIRLVSHPEEPVAGAQTDR
AKLFMVAVKQELSQANFATFTQALQDYKGSDDFAALAAACLGPLFAEDPKKHNL LQGFYQF
VRPHHKQQFEEVC IQL TGRGCGYRPEHSIPRRQRAQPVLDPTGRTAPDPKL TVSTAAQQ
LDPQEHLNQCRPHLSPRPPPTGDPGCSQPQWGSVPRACKQGQHAVSAYLADARRALGSAG
CSQLLAAL TAYKQDDDL DKVLAVLAALTTAKPEDFPL LHRFSMFVRPHHKQRF SQTCTDL
TGRPYPGMEPPGPEERLAVPPVLTHRAPQPGPSRSEKTGKTQSKI SSFLRQRPAGTVGA
GGEDAGPSQSSGPPHGPAASEWGL (SEQ ID NO:2)

NHL
NHL
NHL
NHL
NHL
NHL
NHL

FIG. 3F

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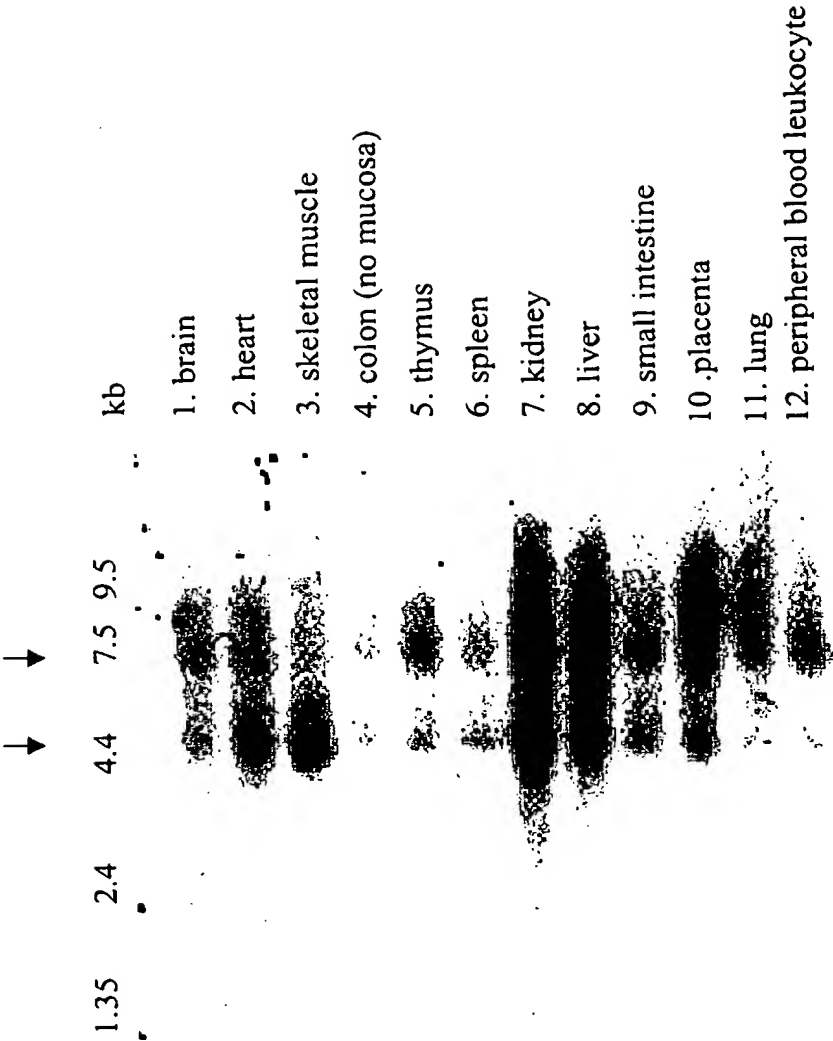


FIG.4

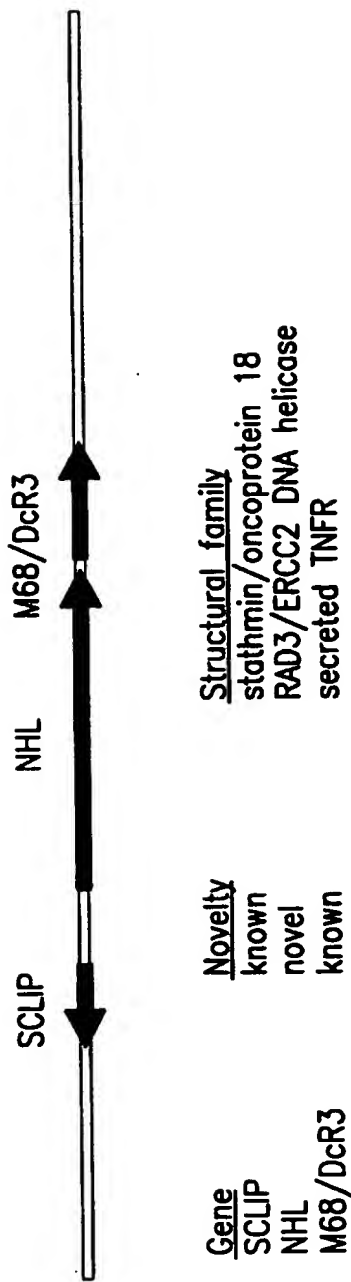


FIG.5B

SEQUENCE LISTING

<110> APPLICANT: Merck & Co., Inc.

<120> TITLE: DNA MOLECULES ENCODING HUMAN NHL, A DNA
HELICASE

<130> DOCKET/FILE REFERENCE: 20585 PCT

<160> NUMBER OF SEQUENCES: 38

<170> SOFTWARE: FastSEQ for Windows Version 4.0

<210> SEQ ID NO:1

<211> LENGTH: 4946

<212> TYPE: DNA

<213> ORGANISM: Homo sapien

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (828)...(4487)

<400> SEQ ID NO:1

```

agtcagccct gctgccagcc agtgccgggt gctggggact cagggaggcc cgccgggacc      60
actgcgggac agtgagccga gcagaagctg gaacgcagga gaggaaggag agggggcggt      120
cagggctctc aggagccggg tcctgggcaa ggcgcagccg ttttcaaatt ttcaggaaag      180
cggtcggctc acactcgagc agtaaaaaga tgcctctggg gagggaggcc gtgcagctct      240
ccgggcaatg gtggtggctc ggcctagaga ggcggtagt gaacgcagac cctggtgggg      300
gaatgacatc aagggaggag acgggcggga cccagattt ctgcctgtgg gcgatggaag      360
tgaggttcac tggccagcgg agccggacac agaacgcgca aaacgccgtg taggcctgga      420
ggagccgaag agcaggcgga cccctccgc gggggaacag tttccgccgg gagcacaag      480
caacggaccg gaagtggggg gcggaagtgc agtgggctca gcgccgactg cgcgcctctg      540
cccgcgaaaa ctctgagctg gctgacagct ggggacgggt ggcggccctc gactggagtc      600
ggttgagttc ctgagggacc ccggttctgg aaggttcgcc gcggagacaa gtgagcagtc      660
tgtgccatag ggattctcga agagaacagc gttgtgtccc agtgcacatg ctgcgcatgc      720
ttaccaggag tgcccagac cctaagatgt tcggagtggg tttttcgcac agaccggaat      780
agcctgcccc tcagccacgc tctgtgccct tctgagaaca ggctgat atg ccc aag      836
                                     Met Pro Lys
                                     1

```

```

ata gtc ctg aat ggt gtg acc gta gac ttc cct ttc cag ccc tac aaa      884
Ile Val Leu Asn Gly Val Thr Val Asp Phe Pro Phe Gln Pro Tyr Lys
   5                10                15

```

```

tgc caa cag gag tac atg acc aag gtc ctg gaa tgt ctg cag cag aag      932
Cys Gln Gln Glu Tyr Met Thr Lys Val Leu Glu Cys Leu Gln Gln Lys
   20                25                30                35

```

```

gtg aat ggc atc ctg gag agc cct acg ggt aca ggg aag acg ctg tgc      980
Val Asn Gly Ile Leu Glu Ser Pro Thr Gly Thr Gly Lys Thr Leu Cys
          40                45                50

```

```

ctg ctg tgc acc acg ctg gcc tgg cga gaa cac ctc cga gac ggc atc      1028
Leu Leu Cys Thr Thr Leu Ala Trp Arg Glu His Leu Arg Asp Gly Ile
          55                60                65

```

```

tct gcc cgc aag att gcc gag agg gcg caa gga gag ctt ttc ccg gat      1076
Ser Ala Arg Lys Ile Ala Glu Arg Ala Gln Gly Glu Leu Phe Pro Asp
          70                75                80

```

cgg gcc ttg tca tcc tgg ggc aac gct gct gct gct gct gga gac ccc	1124
Arg Ala Leu Ser Ser Trp Gly Asn Ala Ala Ala Ala Ala Gly Asp Pro	
85 90 95	
ata gct tgc tac acg gac atc cca aag att att tac gcc tcc agg acc	1172
Ile Ala Cys Tyr Thr Asp Ile Pro Lys Ile Ile Tyr Ala Ser Arg Thr	
100 105 110 115	
cac tcg caa ctc aca cag gtc atc aac gag ctt cgg aac acc tcc tac	1220
His Ser Gln Leu Thr Gln Val Ile Asn Glu Leu Arg Asn Thr Ser Tyr	
120 125 130	
cgg cct aag gtg tgt gtg ctg ggc tcc cgg gag cag ctg tgc atc cat	1268
Arg Pro Lys Val Cys Val Leu Gly Ser Arg Glu Gln Leu Cys Ile His	
135 140 145	
cct gag gtg aag aaa caa gag agt aac cat cta cag atc cac ttg tgc	1316
Pro Glu Val Lys Lys Gln Glu Ser Asn His Leu Gln Ile His Leu Cys	
150 155 160	
cgt aag aag gtg gca agt cgc tcc tgt cat ttc tac aac aac gta gaa	1364
Arg Lys Lys Val Ala Ser Arg Ser Cys His Phe Tyr Asn Asn Val Glu	
165 170 175	
gaa aaa agc ctg gag cag gag ctg gcc agc ccc atc ctg gac att gag	1412
Glu Lys Ser Leu Glu Gln Glu Leu Ala Ser Pro Ile Leu Asp Ile Glu	
180 185 190 195	
gac ttg gtc aag agc gga agc aag cac agg gtg tgc cct tac tac ctg	1460
Asp Leu Val Lys Ser Gly Ser Lys His Arg Val Cys Pro Tyr Tyr Leu	
200 205 210	
tcc cgg aac ctg aag cag caa gcc gac atc ata ttc atg ccg tac aat	1508
Ser Arg Asn Leu Lys Gln Gln Ala Asp Ile Ile Phe Met Pro Tyr Asn	
215 220 225	
tac ttg ttg gat gcc aag agc cgc aga gca cac aac att gac ctg aag	1556
Tyr Leu Leu Asp Ala Lys Ser Arg Arg Ala His Asn Ile Asp Leu Lys	
230 235 240	
ggg aca gtc gtg atc ttt gac gaa gct cac aac gtg gag aag atg tgt	1604
Gly Thr Val Val Ile Phe Asp Glu Ala His Asn Val Glu Lys Met Cys	
245 250 255	
gaa gaa tcg gca tcc ttt gac ctg act ccc cat gac ctg gct tca gga	1652
Glu Glu Ser Ala Ser Phe Asp Leu Thr Pro His Asp Leu Ala Ser Gly	
260 265 270 275	
ctg gac gtc ata gac cag gtg ctg gag gag cag acc aag gca gcg cag	1700
Leu Asp Val Ile Asp Gln Val Leu Glu Glu Gln Thr Lys Ala Ala Gln	
280 285 290	
cag ggt gag ccc cac ccg gag ttc agc gcg gac tcc ccc agc cca ggg	1748
Gln Gly Glu Pro His Pro Glu Phe Ser Ala Asp Ser Pro Ser Pro Gly	
295 300 305	
ctg aac atg gag ctg gaa gac att gca aag ctg aag atg atc ctg ctg	1796
Leu Asn Met Glu Leu Glu Asp Ile Ala Lys Leu Lys Met Ile Leu Leu	
310 315 320	

cgc ctg gag ggg gcc atc gat gct gtt gag ctg cct gga gac gac agc Arg Leu Glu Gly Ala Ile Asp Ala Val Glu Leu Pro Gly Asp Asp Ser 325 330 335	1844
ggc gtc acc aag cca ggg agc tac atc ttt gag ctg ttt gct gaa gcc Gly Val Thr Lys Pro Gly Ser Tyr Ile Phe Glu Leu Phe Ala Glu Ala 340 345 350 355	1892
cag atc acg ttt cag acc aag ggc tgc atc ctg gac tcg ctg gac cag Gln Ile Thr Phe Gln Thr Lys Gly Cys Ile Leu Asp Ser Leu Asp Gln 360 365 370	1940
atc atc cag cac ctg gca gga cgt gct gga gtg ttc acc aac acg gcc Ile Ile Gln His Leu Ala Gly Arg Ala Gly Val Phe Thr Asn Thr Ala 375 380 385	1988
gga ctg cag aag ctg gcg gac att atc cag att gtg ttc agt gtg gac Gly Leu Gln Lys Leu Ala Asp Ile Ile Gln Ile Val Phe Ser Val Asp 390 395 400	2036
ccc tcc gag ggc agc cct ggt tcc cca gca ggg ctg ggg gcc tta cag Pro Ser Glu Gly Ser Pro Gly Ser Pro Ala Gly Leu Gly Ala Leu Gln 405 410 415	2084
tcc tat aag gtg cac atc cat cct gat gct ggt cac cgg agg acg gct Ser Tyr Lys Val His Ile His Pro Asp Ala Gly His Arg Arg Thr Ala 420 425 430 435	2132
cag cgg tct gat gcc tgg agc acc act gca gcc aga aag cga ggg aag Gln Arg Ser Asp Ala Trp Ser Thr Thr Ala Ala Arg Lys Arg Gly Lys 440 445 450	2180
gtg ctg agc tac tgg tgc ttc agt ccc ggc cac agc atg cac gag ctg Val Leu Ser Tyr Trp Cys Phe Ser Pro Gly His Ser Met His Glu Leu 455 460 465	2228
gtc cgc cag ggc gtc cgc tcc ctc atc ctt acc agc ggc acg ctg gcc Val Arg Gln Gly Val Arg Ser Leu Ile Leu Thr Ser Gly Thr Leu Ala 470 475 480	2276
ccg gtg tcc tcc ttt gct ctg gag atg cag atc cct ttc cca gtc tgc Pro Val Ser Ser Phe Ala Leu Glu Met Gln Ile Pro Phe Pro Val Cys 485 490 495	2324
ctg gag aac cca cac atc atc gac aag cac cag atc tgg gtg ggg gtc Leu Glu Asn Pro His Ile Ile Asp Lys His Gln Ile Trp Val Gly Val 500 505 510 515	2372
gtc ccc aga ggc ccc gat gga gcc cag ttg agc tcc gcg ttt gac aga Val Pro Arg Gly Pro Asp Gly Ala Gln Leu Ser Ser Ala Phe Asp Arg 520 525 530	2420
cgg ttt tcc gag gag tgc tta tcc tcc ctg ggg aag gct ctg ggc aac Arg Phe Ser Glu Glu Cys Leu Ser Ser Leu Gly Lys Ala Leu Gly Asn 535 540 545	2468
atc gcc cgc gtg gtg ccc tat ggg ctc ctg atc ttc ttc cct tcc tat Ile Ala Arg Val Val Pro Tyr Gly Leu Leu Ile Phe Phe Pro Ser Tyr 550 555 560	2516

cct gtc atg gag aag agc ctg gag ttc tgg cgg gcc cgc gac ttg gcc Pro Val Met Glu Lys Ser Leu Glu Phe Trp Arg Ala Arg Asp Leu Ala 565 570 575	2564
agg aag atg gag gcg ctg aag ccg ctg ttt gtg gag ccc agg agc aaa Arg Lys Met Glu Ala Leu Lys Pro Leu Phe Val Glu Pro Arg Ser Lys 580 585 590 595	2612
ggc agc ttc tcc gag acc atc agt gct tac tat gca agg gtt gcc gcc Gly Ser Phe Ser Glu Thr Ile Ser Ala Tyr Tyr Ala Arg Val Ala Ala 600 605 610	2660
cct ggg tcc acc ggc gcc acc ttc ctg gcg gtc tgc cgg ggc aag gcc Pro Gly Ser Thr Gly Ala Thr Phe Leu Ala Val Cys Arg Gly Lys Ala 615 620 625	2708
agc gag ggg ctg gac ttc tca gac acg aat ggc cgt ggt gtg att gtc Ser Glu Gly Leu Asp Phe Ser Asp Thr Asn Gly Arg Gly Val Ile Val 630 635 640	2756
acg ggc ctc ccg tac ccc cca cgc atg gac ccc cgg gtt gtc ctc aag Thr Gly Leu Pro Tyr Pro Pro Arg Met Asp Pro Arg Val Val Leu Lys 645 650 655	2804
atg cag ttc ctg gat gag atg aag ggc cag ggt ggg gct ggg ggc cag Met Gln Phe Leu Asp Glu Met Lys Gly Gln Gly Gly Ala Gly Gly Gln 660 665 670 675	2852
ttc ctc tct ggg cag gag tgg tac cgg cag cag gcg tcc agg gct gtg Phe Leu Ser Gly Gln Glu Trp Tyr Arg Gln Gln Ala Ser Arg Ala Val 680 685 690	2900
aac cag gcc atc ggg cga gtg atc cgg cac cgc cag gac tac gga gct Asn Gln Ala Ile Gly Arg Val Ile Arg His Arg Gln Asp Tyr Gly Ala 695 700 705	2948
gtc ttc ctc tgt gac cac agg ttc gcc ttt gcc gac gca aga gcc caa Val Phe Leu Cys Asp His Arg Phe Ala Phe Ala Asp Ala Arg Ala Gln 710 715 720	2996
ctg ccc tcc tgg gtg cgt ccc cac gtc agg gtg tat gac aac ttt ggc Leu Pro Ser Trp Val Arg Pro His Val Arg Val Tyr Asp Asn Phe Gly 725 730 735	3044
cat gtc atc cga gac gtg gcc cag ttc ttc cgt gtt gcc gag cga act His Val Ile Arg Asp Val Ala Gln Phe Phe Arg Val Ala Glu Arg Thr 740 745 750 755	3092
atg cca gcg ccg gcc ccc cgg gct aca gca ccc agt gtg cgt gga gaa Met Pro Ala Pro Ala Pro Arg Ala Thr Ala Pro Ser Val Arg Gly Glu 760 765 770	3140
gat gct gtc agc gag gcc aag tcg cct ggc ccc ttc ttc tcc acc agg Asp Ala Val Ser Glu Ala Lys Ser Pro Gly Pro Phe Phe Ser Thr Arg 775 780 785	3188
aaa gct aag agt ctg gac ctg cat gtc ccc agc ctg aag cag agg tcc Lys Ala Lys Ser Leu Asp Leu His Val Pro Ser Leu Lys Gln Arg Ser 790 795 800	3236

tca ggg tca cca gct gcc ggg gac ccc gag agt agc ctg tgt gtg gag Ser Gly Ser Pro Ala Ala Gly Asp Pro Glu Ser Ser Leu Cys Val Glu 805 810 815	3284
tat gag cag gag cca gtt cct gcc cgg cag agg ccc agg ggg ctg ctg Tyr Glu Gln Glu Pro Val Pro Ala Arg Gln Arg Pro Arg Gly Leu Leu 820 825 830 835	3332
gcc gcc ctg gag cac agc gaa cag cgg gcg ggg agc cct ggc gag gag Ala Ala Leu Glu His Ser Glu Gln Arg Ala Gly Ser Pro Gly Glu Glu 840 845 850	3380
cag gcc cac agc tgc tcc acc ctg tcc ctc ctg tct gag aag agg ccg Gln Ala His Ser Cys Ser Thr Leu Ser Leu Leu Ser Glu Lys Arg Pro 855 860 865	3428
gca gaa gaa ccg cga gga ggg agg aag aag atc cgg ctg gtc agc cac Ala Glu Glu Pro Arg Gly Gly Arg Lys Lys Ile Arg Leu Val Ser His 870 875 880	3476
ccg gag gag ccc gtg gct ggt gca cag acg gac agg gcc aag ctc ttc Pro Glu Glu Pro Val Ala Gly Ala Gln Thr Asp Arg Ala Lys Leu Phe 885 890 895	3524
atg gtg gcc gtg aag cag gag ttg agc caa gcc aac ttt gcc acc ttc Met Val Ala Val Lys Gln Glu Leu Ser Gln Ala Asn Phe Ala Thr Phe 900 905 910 915	3572
acc cag gcc ctg cag gac tac aag ggt tcc gat gac ttc gcc gcc ctg Thr Gln Ala Leu Gln Asp Tyr Lys Gly Ser Asp Asp Phe Ala Ala Leu 920 925 930	3620
gcc gcc tgt ctc ggc ccc ctc ttt gct gag gac ccc aag aag cac aac Ala Ala Cys Leu Gly Pro Leu Phe Ala Glu Asp Pro Lys Lys His Asn 935 940 945	3668
ctg ctc caa ggc ttc tac cag ttt gtg cgg ccc cac cat aag cag cag Leu Leu Gln Gly Phe Tyr Gln Phe Val Arg Pro His His Lys Gln Gln 950 955 960	3716
ttt gag gag gtc tgt atc cag ctg aca gga cga ggc tgt ggc tat cgg Phe Glu Glu Val Cys Ile Gln Leu Thr Gly Arg Gly Cys Gly Tyr Arg 965 970 975	3764
cct gag cac agc att ccc cga agg cag cgg gca cag ccg gtc ctg gac Pro Glu His Ser Ile Pro Arg Arg Gln Arg Ala Gln Pro Val Leu Asp 980 985 990 995	3812
ccc act gga aga acg gcg ccg gat ccc aag ctg acc gtg tcc acg gct Pro Thr Gly Arg Thr Ala Pro Asp Pro Lys Leu Thr Val Ser Thr Ala 1000 1005 1010	3860
gca gcc cag cag ctg gac ccc caa gag cac ctg aac cag ggc agg ccc Ala Ala Gln Gln Leu Asp Pro Gln Glu His Leu Asn Gln Gly Arg Pro 1015 1020 1025	3908
cac ctg tcg ccc agg cca ccc cca aca gga gac cct ggc agc caa cca His Leu Ser Pro Arg Pro Pro Thr Gly Asp Pro Gly Ser Gln Pro 1030 1035 1040	3956

cag tgg ggg tct gga gtg ccc aga gca ggg aag cag ggc cag cac gcc 4004
 Gln Trp Gly Ser Gly Val Pro Arg Ala Gly Lys Gln Gly Gln His Ala
 1045 1050 1055

gtg agc gcc tac ctg gct gat gcc cgc agg gcc ctg ggg tcc gcg ggc 4052
 Val Ser Ala Tyr Leu Ala Asp Ala Arg Arg Ala Leu Gly Ser Ala Gly
 1060 1065 1070 1075

tgt agc caa ctc ttg gca gcg ctg aca gcc tat aag caa gac gac gac 4100
 Cys Ser Gln Leu Leu Ala Ala Leu Thr Ala Tyr Lys Gln Asp Asp Asp
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ctc gac aag gtg ctg gct gtg ttg gcc gcc ctg acc act gca aag cca 4148
 Leu Asp Lys Val Leu Ala Val Leu Ala Ala Leu Thr Thr Ala Lys Pro
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gag gac ttc ccc ctg ctg cac agg ttc agc atg ttt gtg cgt cca cac 4196
 Glu Asp Phe Pro Leu Leu His Arg Phe Ser Met Phe Val Arg Pro His
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cac aag cag cgc ttc tca cag acg tgc aca gac ctg acc ggc cgg ccc 4244
 His Lys Gln Arg Phe Ser Gln Thr Cys Thr Asp Leu Thr Gly Arg Pro
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 Tyr Pro Gly Met Glu Pro Pro Gly Pro Gln Glu Glu Arg Leu Ala Val
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 Pro Pro Val Leu Thr His Arg Ala Pro Gln Pro Gly Pro Ser Arg Ser
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 Glu Lys Thr Gly Lys Thr Gln Ser Lys Ile Ser Ser Phe Leu Arg Gln
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<210> SEQ ID NO:2
 <211> LENGTH: 1219
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapien

<400> SEQ ID NO:2

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Phe	Pro	Asp	Arg	Ala	Leu	Ser	Ser	Trp	Gly	Asn	Ala	Ala	Ala	Ala	Ala
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 Pro Val Cys Leu Glu Asn Pro His Ile Ile Asp Lys His Gln Ile Trp
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 Val Gly Val Val Pro Arg Gly Pro Asp Gly Ala Gln Leu Ser Ser Ala
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 Tyr Gly Ala Val Phe Leu Cys Asp His Arg Phe Ala Phe Ala Asp Ala
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 Arg Gly Glu Asp Ala Val Ser Glu Ala Lys Ser Pro Gly Pro Phe Phe
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 Ser Thr Arg Lys Ala Lys Ser Leu Asp Leu His Val Pro Ser Leu Lys
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 Gln Arg Ser Ser Gly Ser Pro Ala Ala Gly Asp Pro Glu Ser Ser Leu
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 Gly Leu Leu Ala Ala Leu Glu His Ser Glu Gln Arg Ala Gly Ser Pro
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<210> SEQ ID NO:3

<211> LENGTH: 114793

<212> TYPE: DNA

<213> ORGANISM:Homo sapien

<400> SEQ ID NO:3

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catgacgaaa	cccatctcca	ccaaaattac	aaaaaattag	tctggcatgg	tggcacgcgc	112980
ctgtagtccc	agctatttgg	gggaggatcc	cagctaagggt	gggaggatca	cttgagcctg	113040
ggaagtcaag	gctgcagtga	gctgagattg	tgccactgca	ctccagcctg	ggtgcagatc	113100
ttatctcaga	agtaaaggga	ctaggaatgg	tggcttttat	ctctaattccc	agcacttttg	113160
gaggctgagg	tgagtggatc	accggaggtc	aggagtttaa	gaccagcctg	gccaacatgg	113220
tgaacccccg	tctctactaa	aaatacaaaa	agtagccggg	tgtggtggtg	ggtgtctgta	113280
atcccagcta	ctcgggaggc	tgaggcaaga	gaatcgcttg	aacctgggaa	gcggagggtg	113340
cagtgcagca	gatgcacca	ctgcattaca	gcctagatga	cagagcgaga	ctctgcctaa	113400
aaaaaaaaaa	aaaaagaaaa	gaaaagaaat	taagatctag	acactgtggt	tcatgcctgt	113460
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aaaaagtata	cttgtttttt	tgtatcatcc	atattttacc	tttctttttt	ttgccctttt	113640
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tcagcctccc	aagtagctga	gactgcaggc	acctgccacc	acccccagcc	aatttttttg	113880
gtatttttag	tagagacagg	gtttcactat	gttgccagg	ctggtctcga	attcatgacc	113940
tcatgatctg	cctgtcctgg	cctcccaaaa	tgctgggatt	accatgagcc	accacgcca	114000
gcccatttag	aattttcttt	tttttttttt	ttttgagatg	gggtctcgct	cttgtttccc	114060
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catctcctgt	ccagcctc	ccagtagctg	ggcctgcca	ccacgcccac		114180
ctaatttttt	gtatttttag	gagagatggg	gtttcaccat	gttagccagg	atggctctga	114240

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tctcctgacc tcgtgatccg cccgccttgg cctcccaaag tgctgggatt acaggcgtga 114300
gccaccgcgc ccggctagaa tttctttag tagacaggttg ctagcaacca attcagtgtt 114360
tatttgggaa tgtctttatt tcagcttcat tttttgaagg atagtttagc tggctataga 114420
attattaatt gatcattctt ttcagtgttt aaaagtgtca tcatgctacc ttctgggttc 114480
cattgtttct gatgagaagt catctgtcaa attgtccctt tgtacttgaa gaattatctt 114540
tttttctctt gatgttttca agattttctc tttgtctttg gccttttagta gtttgtgatg 114600
tatctagggtg tggatctctt ggtgtgcatc gtatttgggc ttcagtaagc ctcttagatt 114660
catagattaa tgttttgttt tgttttacca aatttggaga gtttttactc atcatttcaa 114720
caaatttttt tcctgcccct ctctcatctc cttttgggag taccactgca tgtatgttgg 114780
tgtgcgttct cta 114793

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<210> SEQ ID NO:4
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

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<400> SEQ ID NO:4
cacaggttca gcatgtttgt gcgtc 25

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<210> SEQ ID NO:5
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

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<400> SEQ ID NO:5
cacagtcctt gctggcctct gtcta 25

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<210> SEQ ID NO:6
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

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<400> SEQ ID NO:6
caggacatct ccatcaagag gctgc 25

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<210> SEQ ID NO:7
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

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<400> SEQ ID NO:7
aataagaggg ggccaggatc agtgc 25

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<210> SEQ ID NO:8
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:8
gtgaatggca tcctggagag 20

<210> SEQ ID NO:9
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:9
gtctccaggc agctcaacag 20

<210> SEQ ID NO:10
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:10
accctgtccc tcctgtctga 20

<210> SEQ ID NO:11
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:11
agaccctaag atgttcggag 20

<210> SEQ ID NO:12
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:12
gatgacctgt gtgagttgcg 20

<210> SEQ ID NO:13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:13
cgcaactcac acaggtcatc 20

<210> SEQ ID NO:14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:14
ggagtcaggt caaaggatgc 20

<210> SEQ ID NO:15
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:15
gcaccccttg acctgactcc 20

<210> SEQ ID NO:16
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:16
ggtctgaaac gtgatctggg 20

<210> SEQ ID NO:17
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:17
cccagatcac gtttcagacc 20

<210> SEQ ID NO:18
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:18
cgatgatgtg tgggttctcc 20

<210> SEQ ID NO:19
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:19
ggagaaccca cacatcatcg 20

<210> SEQ ID NO:20
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:20
cgtgtctgag aagtcagcc 20

<210> SEQ ID NO:21
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:21
ggctggactt ctcagacacg 20

<210> SEQ ID NO:22
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:22
acagcatctt ctccacgcac 20

<210> SEQ ID NO:23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:23
agtcctctgg ctttgcatg 20

<210> SEQ ID NO:24
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:24
tgtgcgtgga gaagatgctg 20

<210> SEQ ID NO:25
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:25
ggctggaaag ggaagtctac 20

<210> SEQ ID NO:26
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:26
tggttcaggt gctcttgggg 20

<210> SEQ ID NO:27
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:27
cgtgaagcag gagttgagcc 20

<210> SEQ ID NO:28
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:28
atcttgctct gggtcttccc 20

<210> SEQ ID NO:29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:29
cactgcaaag ccagaggact 20

<210> SEQ ID NO:30
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:30
ataagcaaga cgacgacctc
20

<210> SEQ ID NO:31
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:31
ctattctggt ggggtgggttc
20

<210> SEQ ID NO:32
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:32
cgtgcctcct gtgcttacct
20

<210> SEQ ID NO:33
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:33
cagaccccaa ggtagctcag
20

<210> SEQ ID NO:34
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM:Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQ ID NO:34
ggaagaccga gagcaagatc
20

<210> SEQ ID NO:35
<211> LENGTH: 780
<212> TYPE: PRT
<213> ORGANISM:Dictyostelium discoideum

<400> SEQ ID NO:35
Met Lys Phe Tyr Ile Glu Asp Leu Leu Val Tyr Phe Pro Tyr Ser Tyr
1           5           10           15
Ile Tyr Pro Glu Gln Tyr Ser Tyr Met Val Ala Leu Lys Arg Ser Leu
20           25           30

```

Asp Asn Gly Gly Pro Cys Ile Leu Glu Met Pro Ser Gly Thr Gly Lys
 35 40 45
 Thr Val Ser Leu Leu Ser Leu Ile Ser Ser Tyr Gln Val Lys Asn Pro
 50 55 60
 Ser Ile Lys Leu Ile Tyr Cys Ser Arg Thr Val Pro Glu Ile Glu Gln
 65 70 75 80
 Ala Thr Glu Glu Ala Arg Arg Val Leu Gln Tyr Arg Asn Ser Glu Met
 85 90 95
 Gly Glu Glu Ser Pro Lys Thr Leu Cys Met Ser Met Ser Ser Arg Arg
 100 105 110
 Asn Leu Cys Ile Gln Pro Arg Val Ser Glu Glu Arg Asp Gly Lys Val
 115 120 125
 Val Asp Ala Leu Cys Arg Glu Leu Thr Ser Ser Trp Asn Arg Glu Ser
 130 135 140
 Pro Thr Ser Glu Lys Cys Lys Phe Phe Glu Asn Phe Glu Ser Asn Gly
 145 150 155 160
 Lys Glu Ile Leu Leu Glu Gly Val Tyr Ser Leu Glu Asp Leu Lys Glu
 165 170 175
 Tyr Gly Leu Lys His Gln Met Cys Pro Tyr Phe Leu Ser Arg His Met
 180 185
 Leu Asn Phe Ala Asn Ile Val Ile Phe Ser Tyr Gln Tyr Leu Leu Asp
 195 200 205
 Pro Lys Ile Ala Ser Leu Ile Ser Ser Ser Phe Pro Ser Asn Ser Ile
 210 215 220
 Val Val Phe Asp Glu Ala His Asn Ile Asp Asn Val Cys Ile Asn Ala
 225 230 235 240
 Leu Ser Ile Asn Ile Asp Asn Lys Leu Leu Asp Thr Ser Ser Lys Asn
 245 250 255
 Ile Ala Lys Ile Asn Lys Gln Ile Glu Asp Ile Lys Lys Val Asp Glu
 260 265 270
 Lys Arg Leu Lys Asp Glu Tyr Gln Arg Leu Val Asn Gly Leu Ala Arg
 275 280 285
 Ser Gly Ser Thr Arg Ala Asp Glu Thr Thr Ser Asp Pro Val Leu Pro
 290 295 300
 Asn Asp Val Ile Gln Glu Ala Val Pro Gly Asn Ile Arg Lys Pro Ser
 305 310 315 320
 Ile Phe Ile Ser Leu Leu Arg Arg Val Val Asp Tyr Leu Arg Glu Pro
 325 330 335
 Asp Lys Ser Arg Leu Lys Ser Gln Met Leu Leu Ser Glu Ser Pro Leu
 340 345 350
 Ala Phe Leu Gln Gly Leu Tyr His Ala Thr Gln Ile Ser Ser Arg Thr
 355 360 365
 Leu Arg Phe Cys Ser Ser Arg Leu Ser Ser Leu Leu Arg Thr Leu Arg
 370 375 380
 Ile Asn Asp Val Asn Gln Phe Ser Gly Ile Ser Leu Ile Ala Asp Phe
 385 390 395 400
 Ala Thr Leu Val Gly Thr Tyr Asn Asn Gly Phe Leu Ile Ile Ile Glu
 405 410 415
 Pro Tyr Tyr Gln Arg Gln Asn Asn Thr Tyr Asp Gln Ile Phe Gln Phe
 420 425 430
 Cys Cys Leu Asp Ala Ser Ile Gly Met Lys Pro Ile Phe Asp Lys Tyr
 435 440 445
 Arg Ser Val Val Ile Thr Ser Gly Thr Leu Ser Pro Leu Asp Ile Tyr
 450 455 460
 Thr Lys Met Leu Asn Phe Arg Pro Thr Val Val Glu Arg Leu Thr Met
 465 470 475 480
 Ser Leu Asn Arg Asn Cys Ile Cys Pro Cys Ile Leu Thr Arg Gly Ser
 485 490 495
 Asp Gln Ile Ser Ile Ser Thr Lys Phe Asp Val Arg Ser Asp Thr Ala
 500 505 510

```

Val Val Arg Asn Tyr Gly Ala Leu Leu Val Glu Val Ser Ala Ile Val
    515                      520                      525
Pro Asp Gly Ile Ile Cys Phe Phe Thr Ser Tyr Ser Tyr Met Glu Gln
    530                      535                      540
Ile Val Ser Val Trp Asn Glu Met Gly Leu Leu Asn Asn Ile Leu Thr
545                      550                      555                      560
Asn Lys Leu Ile Phe Val Glu Thr Ser Asp Pro Ala Glu Ser Ala Leu
    565                      570                      575
Ala Leu Gln Asn Tyr Lys Lys Ala Cys Asp Ser Gly Arg Gly Ala Val
    580                      585                      590
Leu Leu Ser Val Ala Arg Gly Lys Val Ser Glu Gly Ile Asp Phe Asp
    595                      600                      605
Asn Gln Tyr Gly Arg Cys Val Ile Leu Tyr Gly Ile Pro Tyr Ile Asn
    610                      615                      620
Thr Glu Ser Lys Val Leu Arg Ala Arg Leu Glu Phe Leu Arg Asp Arg
625                      630                      635                      640
Tyr Gln Ile Arg Glu Asn Glu Phe Leu Thr Phe Asp Ala Met Arg Thr
    645                      650                      655
Ala Ser Gln Cys Val Gly Arg Val Ile Arg Gly Lys Ser Asp Tyr Gly
    660                      665                      670
Ile Met Ile Phe Ala Asp Lys Arg Tyr Asn Arg Leu Asp Lys Arg Asn
    675                      680                      685
Lys Leu Pro Gln Trp Ile Leu Gln Phe Cys Gln Pro Gln His Leu Asn
    690                      695                      700
Leu Ser Thr Asp Met Ala Ile Ser Leu Ser Lys Thr Phe Leu Arg Glu
705                      710                      715                      720
Met Gly Gln Pro Phe Ser Arg Glu Glu Gln Leu Gly Lys Ser Leu Trp
    725                      730                      735
Ser Leu Glu His Val Glu Lys Gln Ser Thr Ser Lys Pro Pro Gln Gln
    740                      745                      750
Gln Asn Ser Ala Ile Asn Ser Thr Ile Thr Thr Ser Thr Thr Thr Thr
    755                      760                      765
Thr Thr Thr Ser Thr Ile Ser Glu Thr His Leu Thr
    770                      775                      780

```

<210> SEQ ID NO:36

<211> LENGTH: 778

<212> TYPE: PRT

<213> ORGANISM: *S. cerevisiae*

<400> SEQ ID NO:36

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Met Lys Phe Tyr Ile Asp Asp Leu Pro Val Leu Phe Pro Tyr Pro Lys
 1                      5                      10                      15
Ile Tyr Pro Glu Gln Tyr Asn Tyr Met Cys Asp Ile Lys Lys Thr Leu
    20                      25                      30
Asp Val Gly Gly Asn Ser Ile Leu Glu Met Pro Ser Gly Thr Gly Lys
    35                      40                      45
Thr Val Ser Leu Leu Ser Leu Thr Ile Ala Tyr Gln Met His Tyr Pro
    50                      55                      60
Glu His Arg Lys Ile Ile Tyr Cys Ser Arg Thr Met Ser Glu Ile Glu
65                      70                      75                      80
Lys Ala Leu Val Glu Leu Glu Asn Leu Met Asp Tyr Arg Thr Lys Glu
    85                      90                      95
Leu Gly Tyr Gln Glu Asp Phe Arg Gly Leu Gly Leu Thr Ser Arg Lys
    100                      105                      110
Asn Leu Cys Leu His Pro Glu Val Ser Lys Glu Arg Lys Gly Thr Val
    115                      120                      125
Val Asp Glu Lys Cys Arg Arg Met Thr Asn Gly Gln Ala Lys Arg Lys
    130                      135                      140
Leu Glu Glu Asp Pro Glu Ala Asn Val Glu Leu Cys Glu Tyr His Glu

```

145		150		155		160									
Asn	Leu	Tyr	Asn	Ile	Glu	Val	Glu	Asp	Tyr	Leu	Pro	Lys	Gly	Val	Phe
			165						170					175	
Ser	Phe	Glu	Lys	Leu	Leu	Lys	Tyr	Cys	Glu	Glu	Lys	Thr	Leu	Cys	Pro
			180					185						190	
Tyr	Phe	Ile	Val	Arg	Arg	Met	Ile	Ser	Leu	Cys	Asn	Ile	Ile	Ile	Tyr
			195				200					205			
Ser	Tyr	His	Tyr	Leu	Leu	Asp	Pro	Lys	Ile	Ala	Glu	Arg	Val	Ser	Asn
			210			215					220				
Glu	Val	Ser	Lys	Asp	Ser	Ile	Val	Ile	Phe	Asp	Glu	Ala	His	Asn	Ile
225					230					235					240
Asp	Asn	Val	Cys	Ile	Glu	Ser	Leu	Ser	Leu	Asp	Leu	Thr	Thr	Asp	Ala
				245						250					255
Leu	Arg	Arg	Ala	Thr	Arg	Gly	Ala	Asn	Ala	Leu	Asp	Glu	Arg	Ile	Ser
			260					265						270	
Glu	Val	Arg	Lys	Val	Asp	Ser	Gln	Lys	Leu	Gln	Asp	Glu	Tyr	Glu	Lys
			275				280					285			
Leu	Val	Gln	Gly	Leu	His	Ser	Ala	Asp	Ile	Leu	Thr	Asp	Gln	Glu	Glu
			290			295						300			
Pro	Phe	Val	Glu	Thr	Pro	Val	Leu	Pro	Gln	Asp	Leu	Leu	Thr	Glu	Ala
305					310					315					320
Ile	Pro	Gly	Asn	Ile	Arg	Arg	Ala	Glu	His	Phe	Val	Ser	Phe	Leu	Lys
			325						330					335	
Arg	Leu	Ile	Glu	Tyr	Leu	Lys	Thr	Arg	Met	Lys	Val	Leu	His	Val	Ile
			340					345						350	
Ser	Glu	Thr	Pro	Lys	Ser	Phe	Leu	Gln	His	Leu	Lys	Gln	Leu	Thr	Phe
			355				360					365			
Ile	Glu	Arg	Lys	Pro	Leu	Arg	Phe	Cys	Ser	Glu	Arg	Leu	Ser	Leu	Leu
			370			375					380				
Val	Arg	Thr	Leu	Glu	Val	Thr	Glu	Val	Glu	Asp	Phe	Thr	Ala	Leu	Lys
385					390					395					400
Asp	Ile	Ala	Thr	Phe	Ala	Thr	Leu	Ile	Ser	Thr	Tyr	Glu	Glu	Gly	Phe
				405					410					415	
Leu	Leu	Ile	Ile	Glu	Pro	Tyr	Glu	Ile	Glu	Asn	Ala	Ala	Val	Pro	Asn
			420					425						430	
Pro	Ile	Met	Arg	Phe	Thr	Cys	Leu	Asp	Ala	Ser	Ile	Ala	Ile	Lys	Pro
			435				440					445			
Val	Phe	Glu	Arg	Phe	Ser	Ser	Val	Ile	Ile	Thr	Ser	Gly	Thr	Ile	Ser
			450			455					460				
Pro	Leu	Asp	Met	Tyr	Pro	Arg	Met	Leu	Asn	Phe	Lys	Thr	Val	Leu	Gln
465					470					475					480
Lys	Ser	Tyr	Ala	Met	Thr	Leu	Ala	Lys	Lys	Ser	Phe	Leu	Pro	Met	Ile
				485					490					495	
Ile	Thr	Lys	Gly	Ser	Asp	Gln	Val	Ala	Ile	Ser	Ser	Arg	Phe	Glu	Ile
			500					505					510		
Arg	Asn	Asp	Pro	Ser	Ile	Val	Arg	Asn	Tyr	Gly	Ser	Met	Leu	Val	Glu
			515				520					525			
Phe	Ala	Lys	Ile	Thr	Pro	Asp	Gly	Met	Val	Val	Phe	Phe	Pro	Ser	Tyr
			530			535					540				
Leu	Tyr	Met	Glu	Ser	Ile	Val	Ser	Met	Trp	Gln	Thr	Met	Gly	Ile	Leu
545					550					555					560
Asp	Glu	Val	Trp	Lys	His	Lys	Leu	Ile	Leu	Val	Glu	Thr	Pro	Asp	Ala
				565					570					575	
Gln	Glu	Thr	Ser	Leu	Ala	Leu	Glu	Thr	Tyr	Arg	Lys	Ala	Cys	Ser	Asn
			580					585					590		
Gly	Arg	Gly	Ala	Ile	Leu	Leu	Ser	Val	Ala	Arg	Gly	Lys	Val	Ser	Glu
			595				600					605			
Gly	Ile	Asp	Phe	Asp	His	Gln	Tyr	Gly	Arg	Thr	Val	Leu	Met	Ile	Gly
					615						620				

```

Ile Pro Phe Gln Tyr Thr Glu Ser Arg Ile Leu Lys Ala Arg Leu Glu
625                               630                               635                               640
Phe Met Arg Glu Asn Tyr Arg Ile Arg Glu Asn Asp Phe Leu Ser Phe
                               645                               650                               655
Asp Ala Met Arg His Ala Ala Gln Cys Leu Gly Arg Val Leu Arg Gly
                               660                               665                               670
Lys Asp Asp Tyr Gly Val Met Val Leu Ala Asp Arg Arg Phe Ser Arg
                               675                               680                               685
Lys Arg Ser Gln Leu Pro Lys Trp Ile Ala Gln Gly Leu Ser Asp Ala
                               690                               695                               700
Asp Leu Asn Leu Ser Thr Asp Met Ala Ile Ser Asn Thr Lys Gln Phe
705                               710                               715                               720
Leu Arg Thr Met Ala Gln Pro Thr Asp Pro Lys Asp Gln Glu Gly Val
                               725                               730                               735
Ser Val Trp Ser Tyr Glu Asp Leu Ile Lys His Gln Asn Ser Arg Lys
                               740                               745                               750
Asp Gln Gly Gly Phe Ile Glu Asn Glu Asn Lys Glu Gly Glu Gln Asp
                               755                               760                               765
Glu Asp Glu Asp Glu Asp Ile Glu Met Gln
770                               775

```

<210> SEQ ID NO:37

<211> LENGTH: 772

<212> TYPE: PRT

<213> ORGANISM: *S. pombe*

<400> SEQ ID NO:37

```

Met Lys Phe Tyr Ile Asp Asp Leu Pro Ile Leu Phe Pro Tyr Pro Arg
1                               5                               10                               15
Ile Tyr Pro Glu Gln Tyr Gln Tyr Met Cys Asp Leu Lys His Ser Leu
                               20                               25                               30
Asp Ala Gly Gly Ile Ala Leu Leu Glu Met Pro Ser Gly Thr Gly Lys
                               35                               40                               45
Thr Ile Ser Leu Leu Ser Leu Ile Val Ser Tyr Gln Gln His Tyr Pro
50                               55                               60
Glu His Arg Lys Leu Ile Tyr Cys Ser Arg Thr Met Ser Glu Ile Asp
65                               70                               75                               80
Lys Ala Leu Ala Glu Leu Lys Arg Leu Met Ala Tyr Arg Thr Ser Gln
                               85                               90                               95
Leu Gly Tyr Glu Glu Pro Phe Leu Gly Leu Gly Leu Thr Ser Arg Lys
                               100                              105                              110
Asn Leu Cys Leu His Pro Ser Val Arg Arg Glu Lys Asn Gly Asn Val
115                              120                              125
Val Asp Ala Arg Cys Arg Ser Leu Thr Ala Gly Phe Val Arg Glu Gln
130                              135                              140
Arg Leu Ala Gly Met Asp Val Pro Thr Cys Glu Phe His Asp Asn Leu
145                              150                              155                              160
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Ser Lys Asp Cys Ile Val Val Phe Asp Glu Ala His Asn Ile Asp Asn
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<210> SEQ ID NO:38

<211> LENGTH: 760

<212> TYPE: PRT

<213> ORGANISM:Homo sapien

<400> SEQ ID NO:38

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 Thr Val Ser Leu Leu Ala Leu Ile Met Ala Tyr Gln Arg Ala Tyr Pro
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 Gln Glu Gly Glu Lys Leu Pro Phe Leu Gly Leu Ala Leu Ser Ser Arg
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33065**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12N 9/00, 9/10, 1/20; C12N 15/00; C07H 21/02, 21/04

US CL : 435/183, 193, 252.3, 320.1, 6; 536/23.1, 23.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/183, 193, 252.3, 320.1, 6; 536/23.1, 23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, STN, Medline, CAPLUS, BIOSIS, JAPIO, PATOSWO, PATOSEP, SCISEARCH, EMBASE, search terms, helicase, NHL protein, mammalian, human, RAD3/ERCC2 gene family, SEQ ID NOs : 1 and 2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,843,737 A (CHEN et al) 01 December 1998, see entire document.	1
X, P	BAI et al, Overexpression of M68/DcR3 in human gastrointestinal tract tumors independent of gene amplification and its location in four-gene cluster. Proc. Natl. Acad. Sci. USA. 01 February 2000. Vol 97. No. 3, pages 1230-1235.	1-26
X	US 5,888,792 A (BANDMAN et al) 30 March 1999, see entire document.	1
Y, P	ZHOU et al. Pif1p Helicase, a Catalytic Inhibitor of Telomerase in Yeast. Science. 04 August 2000. Vol-289. pages 771-774.	1

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 MARCH 2001

Date of mailing of the international search report

19 APR 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231

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Authorized Officer

PONNATHAPURA ACHUTAMURTHY

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33065

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,466,576 A (SCHULZ et al) 14 November 1995, see entire document.	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33065

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/33065

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-22 and 26, drawn to a purified DNA molecule encoding a mammalian NHL protein, vectors and host cells comprising said DNA, methods of expressing said DNA and the NHL protein.

Group II, claim(s) 23-25, drawn to an isolated molecule which comprises the nucleotide sequence as set forth in SEQ ID NO: 3.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical relationship shared between the claims of groups I and II corresponds to a DNA molecule encoding a mammalian NHL (novel helicase-like) protein. Chen et al. (US Patent No: 5,843,737) teach a gene that encodes a multifunctional protein having helicase activity and hence the inventions do not share a special technical feature.